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Domestic Sewage Wastewater Irrigation Effects on Soil Fertility, Heavy Metals and Nutritional Quality of Sorghum (*Sorghum bicolor*) Grains

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Abstract

Freshwater scarcity has increased reliance on domestic sewage wastewater for irrigation in arid and semi-arid regions. This study examined the impact of irrigation with sewage wastewater and well water on soil properties and sorghum (*Sorghum bicolor*) grain quality in semi-urban and rural agricultural fields of Coimbatore, India. The physicochemical parameters of the domestic wastewater and well-water irrigation samples were within the permissible limits set by WHO and FAO standards. Well water and Wastewater irrigation improved soil fertility, increasing macronutrients such as nitrogen (176–238 kg/ha), phosphorus (10–29 kg/ha), potassium (471–904 kg/ha), and total organic carbon (0.79–2.3%). Both raw and boiled sorghum grain samples from fields irrigated with well water and domestic sewage wastewater were good sources of protein, carbohydrates, energy, bioactive phenolic substances, and antioxidant activity. The slightly elevated Cd, Pb and Cr concentrations observed in soil and grains were within permissible limits, which highlight food safety concerns.

Keywords: *Sorghum bicolor*; Domestic sewage wastewater; Sustainable agriculture; Water reuse; Mineral and proximate composition.

Introduction

Freshwater scarcity has become one of the most pressing global challenges of the 21st century, exacerbated by climate change, rapid urbanisation, population growth, and increasing agricultural demands (Salakinkop and Hunshal, 2014; Carvalho et al., 2021). Agriculture is the backbone of food security, which accounts for nearly 70% of global freshwater withdrawals, creating immense pressure on already stressed conventional water sources (Elgallal et al., 2015). The volume of wastewater generated from domestic, industrial, and commercial sources has increased substantially due to rapid population growth, urbanisation, improved living standards, and economic development. Concurrently, the productive reuse of wastewater has expanded, particularly in urban and peri-urban areas of developing countries, where millions of small-scale farmers rely on wastewater or wastewater-contaminated water sources to irrigate high-value food crops for urban markets, often in the absence of alternative irrigation supplies. However, wastewater may contain undesirable constituents, including heavy metals, pathogens, and organic pollutants, which pose potential risks to human health, soil quality, and the surrounding environment if not properly managed (Quadir et al., 2010). In water-limited regions, reliance on freshwater for irrigation threatens both crop sustainability and resource viability. As a solution, treated or diluted domestic sewage wastewater (DSW) is increasingly recognised as a cost-effective, eco-friendly, and sustainable alternative, especially in arid and semi-arid areas of India (Ungureanu et al., 2020; Bansod et al., 2022).



Using wastewater treatment plant effluents in agriculture and green landscapes offers affordability, reliable supply, reduced freshwater use, and lower treatment costs (Ganji et al., 2024; Siddhuraju et al., 2025). It addresses rising water demand and limits effluent discharge while supplying nutrients (N, P, K) that enhance soil fertility, reduce fertiliser needs, and support soil microbial activity (Silva, 2023; Mohanty and Das, 2023). This recycling improves organic matter, sustains crop production, and mitigates eutrophication (Heyde et al., 2025; Roopika et al., 2024). While wastewater reuse offers benefits, long-term irrigation can alter soil properties, lowering pH, increasing salinity, sodicity, and heavy metal accumulation (Cd, Pb, Cr), which threaten soil health, crop quality, and food safety (Chaganti et al., 2020; Agrawal et al., 2014). Studies in India report elevated heavy metals and reduced crude fibre in wastewater-irrigated crops (Mohanty and Das, 2023; Najam and Kaur, 2016). India generates ~38,354 million litres of sewage (Bansod et al., 2022), much of it underutilised, with urban and peri-urban agriculture, including Coimbatore, relying heavily on it. Recent initiatives such as Swachh Bharat Mission (SBM), Jal Shakti Abhiyan (JSA) and Jal Jeevan Mission (JJM-U) have improved sewage and wastewater treatment, substantially reduced pollutant loads and enhanced prospects for safe reuse (Mhaske, 2016; Roopika et al., 2024).

Sorghum (*Sorghum bicolor* (L.) Moench), the world's fifth most important cereal, is a resilient C₄ crop capable of thriving under drought, salinity, and high-temperature stress (Paiva et al., 2015; Wang et al., 2024). It plays a vital role in food and nutritional security, especially in marginal environments where water scarcity limits the cultivation of other cereals. In Tamil Nadu and other water-stressed regions of India, sorghum is increasingly cultivated using non-conventional water sources, including sewage wastewater (Alghobar and Suresha, 2017). Its nutritional composition-rich in iron (3-4.5 mg/100 g), proteins, dietary fibre, and bioactive compounds makes it particularly important in combating malnutrition (Tan et al., 2024). Previous studies have indicated that DSW irrigation can enhance sorghum productivity by increasing nutrient uptake and biomass accumulation. For instance, Serraye et al. (2025) reported a 21- 46% increase in sorghum biomass under wastewater irrigation, along with improved nitrogen and phosphorus uptake. Similarly, research in Indian contexts has shown 12–15% higher crude protein content in sorghum grains irrigated with domestic sewage wastewater when compared to those irrigated with well water (Najam and Kaur, 2016; Gourkhede et al., 2026). However, potential trade-offs exist, such as heavy metal accumulation in grains and reductions in crude fibre content, which may compromise nutritional quality (Agrawal et al., 2014).

Although wastewater irrigation presents notable agronomic and economic benefits, its long-term impacts on soil quality, grain nutrient composition, and food safety are not yet fully understood, particularly within semi-urban agricultural landscapes in India. There remains a pressing need to balance the productivity gains of wastewater irrigation with the associated environmental and health risks. Against this backdrop, the present study investigates the comparative effects of diluted domestic sewage wastewater and well water irrigation on soil physicochemical properties and the nutritional and heavy metal composition of sorghum grains in semi-urban and rural agro fields of Coimbatore, Tamil Nadu. The outcomes of this study are expected to provide valuable insights into the sustainable use of domestic wastewater for irrigation, with implications for enhancing food production while ensuring environmental safety and public health.

Materials and Methods

Study Area

Coimbatore, in Tamil Nadu, lies on the Noyyal River at 11°1'6"N, 76°58'21"E, with an elevation of 411 m and an area of 642.12 km², ranking as India's sixteenth-largest urban agglomeration. For this study, semi-urban fields in Madukkarai and Kinathukadavu (cereals irrigated with diluted domestic sewage wastewater) have been irrigated continuously with diluted domestic sewage wastewater for over 5 years as a standard local practice for these perennial agricultural fields. Rural fields in Ettimadai and Kannammanayakkanur (cereals irrigated with well water) have similarly received well water irrigation throughout their cultivation history.

Plant Sampling Details and Processing

Sorghum (*Sorghum bicolor* (L.) Moench), a major cereal in Coimbatore, was chosen for this study. Sorghum grain samples irrigated with domestic sewage wastewater (semi-urban fields) and well water (rural fields) were harvested in August 2018. Generally, harvesting occurs 100 to 120 days after sowing the seeds. The sorghum seeds were divided into raw and boiled portions, the latter prepared by boiling at 100°C for 15 min (water-to-grain ratio 10:1, v/w). After discarding the residual water, samples were oven-dried at 45°C to remove moisture, ground to a fine powder (~0.25 nm) using a Mixer Mill MM400 (Retsch, Germany), and stored in airtight containers at room temperature until further analysis.

Soil and Water Sampling

Soil Sampling and Analysis

Soil samples were collected once, at post-harvest in August 2018, coinciding with sorghum grain harvesting (100–120 days after sowing). No repeated sampling was performed during the growing season, as the study focused on cumulative effects from long-term irrigation practices on mature crop fields. Soils irrigated with domestic sewage

wastewater and well water were collected from respective agricultural fields. After removing surface litter, uniform slices (1–2 cm thick) were obtained to the plough depth (15 cm) using a V-shaped spade cut. Samples from different locations were pooled into a composite unit, mixed, spread, and quartered until ~0.5 kg was obtained. Stones, pebbles, and visible debris were removed. The samples were air-dried, ground, sieved (2 mm), and stored in screw-capped glass bottles at ambient conditions. Physicochemical parameters, including lime status, soil texture/type, pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (N), available phosphorus (P), and exchangeable potassium (K), were analysed following the methods described by Motsara (2015).

Water Sampling and Analysis

Domestic sewage wastewater and well water were collected in pre-cleaned 100 mL polyethylene bottles and transported in insulated containers with ice packs. Physical parameters (pH, EC, BOD, COD, DO, total solids, TSS, TDS, ORP, hardness, alkalinity) and chemical parameters (chloride, sulphate, nitrate, phosphate) were analysed as per APHA/AWWA/WEF (2017) standard methods. Microbiological examinations of domestic sewage wastewater and well water samples were conducted in accordance with internationally recognised standards to ensure the validity, reliability, and comparability of the results. Total coliform bacteria (TCB) and faecal coliform bacteria (FCB) were enumerated using the Most Probable Number (MPN) method as described in the Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2017).

Macro-Micro Elements and Heavy Metal Analysis

Qualitative and quantitative estimation of macroelements, microelements, and heavy metals was performed on soil, sewage wastewater, well water, and raw and processed sorghum grains following acid digestion. Samples were digested using a triple acid mixture of HNO_3 : H_2SO_4 : HClO_4 (9: 2: 1) and diluted to volume with HPLC-grade water (Fisher Chemicals, India). Analytical-grade reagents and HPLC-grade water were used for all dilutions and calibration standards.

Mineral profiling was determined using a PerkinElmer (USA) NexION 300X ICP-Q-MS, following the method described by Del Mastro et al. (2015) with slight modifications in digestion methods (use of triple acid mixture HNO_3 : H_2SO_4 : HClO_4 , 9: 2: 1), employing 99.99% pure argon gas. Elemental standard solutions (100 mg/L; PerkinElmer Pure and Pure Plus) were serially diluted daily to prepare working standards. All standards and digested samples were stored in pre-cleaned polyethylene bottles at 4°C until analysis.

Proximate Composition Analysis

Moisture content of raw and processed *Sorghum bicolor* grains was determined using a Moisture Analyser (Model MA35, Sartorius AG, Germany) at 105°C. Crude lipid was estimated with a SOCS PLUS automatic solvent extractor (Pelican Equipments, India), while crude fibre and ash were measured gravimetrically following AOAC (1990) protocols. Total nitrogen was determined by the Micro-Kjeldahl method using a Kel Plus Nitrogen Analyser (Pelican Equipments, India), and crude protein was calculated using a conversion factor of 6.25 ($\text{N} \times 6.25$). Crude carbohydrate, expressed as nitrogen-free extractives (NFE), was estimated by difference, subtracting the sum of moisture, protein, lipid, fibre, and ash from 100. All proximate values were expressed on a dry matter basis (g/100 g DM). Gross energy (kJ) was calculated using conversion factors: 16.7 kJ/g for protein, 37.7 kJ/g for lipid and 16.7 kJ/g for carbohydrate (NFE) (Siddhuraju et al., 1996).

Solvent Extraction of Total Phenolics

Powdered *Sorghum bicolor* grain samples (15 g) were defatted with petroleum ether (1:10, w/v) under intermittent shaking at room temperature for 24 h, filtered through Whatman No. 1 paper, and air-dried to remove residual solvent. The residues were extracted with 80% acetone (1:8, w/v) by occasional stirring for 48 h at room temperature, followed by filtration. A second extraction was performed with 80% acetone (1:5, w/v) for 24 h under the same conditions. Filtrates were pooled, and solvents were evaporated under reduced pressure at 55 °C using a rotary vacuum evaporator. Extract recovery (%) was calculated from the difference in container weights before and after collection.

Estimation of Total Phenolics and Tannins

Total phenolic content of *Sorghum bicolor* grain extracts was determined by the Folin–Ciocalteu method (Makkar et al., 2007; Siddhuraju and Becker, 2003). Briefly, 100 µL of extract was diluted to 1 mL with distilled water, mixed with 0.5 mL of Folin–Ciocalteu reagent (1:1, v/v) and 2.5 mL of 20% sodium carbonate, vortexed, and incubated in the dark for 40 min at room temperature. Absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Cyber Lab, USA). Analyses were run in triplicate, and results were expressed as tannic acid equivalents (TAE, g/100 g DM).

Tannin content was estimated by the PVPP precipitation method described by Makkar et al. (2007) and Siddhuraju and Manian (2007). The PVPP (100 mg) was mixed with 1 mL distilled water and 1 mL extracts, vortexed, and

incubated at 4 °C for 4 h. After centrifugation (3000 ×g, 10 min), the supernatant (non-tannin phenolics) was assayed by the Folin-Ciocalteu method. Tannin content (%) was calculated as:

$$\text{Tannin content (\%)} = \text{Total phenolics (\%)} - \text{Non-tannin phenolics (\%)}$$

Total Flavonoids

Total flavonoid content of *Sorghum bicolor* grain sample extracts was determined spectrophotometrically following the method outlined by Siddhuraju and Becker (2003). An aliquot of 0.1 mL of sample or rutin standard (0–100 mg/L, n = 6) was added to 4 mL of distilled water in a 10 mL volumetric flask. Then, 0.3 mL of 5% NaNO₂ was added; after 5 min, 3 mL of 10% AlCl₃ was added, followed by 2 mL of 1 M NaOH at 6 min. The mixture was diluted to 10 mL, mixed, and absorbance was measured at 510 nm against a reagent blank using a UV-Vis spectrophotometer. Total flavonoid content was calculated from the rutin standard curve and expressed as mg rutin equivalents (RUT) per gram of extract (dry matter basis).

Antioxidant Activity

Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of raw and processed *Sorghum bicolor* grain extracts was measured using the Ferric Reducing Antioxidant Power (FRAP) assay (Siddhuraju and Manian, 2007). Briefly, 900 µL of freshly prepared FRAP reagent (pre-incubated at 37 °C) was mixed with 90 µL of distilled water and 30 µL of sample or methanol (blank), then incubated at 37 °C for 30 min. FRAP reagent consisted of 2.5 mL 20 mmol/L TPTZ in 40 mmol/L HCl, 2.5 mL 20 mmol/L FeCl₃·6H₂O, and 25 mL 0.3 mol/L acetate buffer (pH 3.6). Absorbance was measured at 593 nm using a UV-Vis spectrophotometer. A calibration curve was prepared using FeSO₄·7H₂O (100–2000 µmol/L), and antioxidant activity was expressed as Equivalent Concentration (EC₁), defined as the concentration of sample producing ferric-TPTZ reducing power equivalent to 1 mmol/L Fe²⁺, calculated from the standard curve.

Metal Chelating Activity

The ferrous ion-chelating activity of *Sorghum bicolor* grain extracts was determined following the method of Dinis et al. (1994) with slight modifications. Briefly, 100 µL of extract was mixed with 50 µL of 2 mmol/L FeCl₂, followed by 200 µL of 5 mmol/L ferrozine. The mixture was vortexed and incubated at room temperature for 10 min. Absorbance was measured at 562 nm using a UV-Vis spectrophotometer. Chelating activity was quantified using a calibration curve of EDTA (0.5–2.5 µg) and expressed as mg EDTA equivalents per gram of extract (mg EDTA eq/g).

Stable Free Radical Scavenging Activity Using DPPH• Method

Free radical scavenging activity of *Sorghum bicolor* grain extracts was measured using the DPPH• assay (Brand-Williams et al., 1995; Dhanya et al., 2020). Briefly, 0.1 mL of methanolic extract was mixed with 3.9 mL of 6 × 10⁻⁵ M DPPH• solution and incubated in the dark at room temperature for 30 min. The decrease in absorbance was measured at 515 nm using a UV-Vis spectrophotometer. Antioxidant activity was quantified from a Trolox standard curve and expressed as mmol Trolox equivalents per gram of extract (mmol TE/g).

Total Antioxidant Activity by ABTS•+ Cation Radical Scavenging Assay

The ABTS•+ scavenging activity of *Sorghum bicolor* extracts was determined following Siddhuraju and Becker (2003). First, ABTS•+ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark for 12–16 h. The solution was then diluted with ethanol (~1:89, v/v) and equilibrated to 30 °C to an absorbance of 0.700 ± 0.02 at 734 nm. Next, a 10 µL aliquot of extract or Trolox standard (0–15 µM) was mixed with 1 mL of ABTS•+ solution and incubated at 30 °C for 30 min. Absorbance was measured at 734 nm using a UV-Vis spectrophotometer, with ethanol as the blank. All measurements were performed in triplicate, and results were expressed as µmol Trolox equivalents per gram of extract (µmol TE/g) using a Trolox calibration curve.

Hydroxyl Radical Scavenging Activity (HRSA)

The HRSA of raw and processed *Sorghum bicolor* grain extracts was measured following the method of Klein et al. (1981), with slight modifications by Nagarani et al. (2014). Respective raw and processed extracts (200 µg) were added to 1 mL iron-EDTA solution (0.13% ferrous ammonium sulfate, 0.26% EDTA), 0.5 mL 0.018% EDTA, and 1 mL dimethyl sulfoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated with 0.5 mL of 0.22% ascorbic acid and incubated at 80–90 °C for 15 min. After incubation, 1 mL of ice-cold 17.5% TCA was added, followed by 3 mL of Nash reagent, and the mixture was incubated at room temperature for 15 min. Absorbance of the yellow chromophore was measured at 412 nm using a UV-Vis spectrophotometer, with a control lacking the extract. The hydroxyl radical scavenging activity was calculated using the formula given below:

$$(\%) \text{ HRSA} = 1 - (\text{difference in absorbance of sample} / \text{difference in absorbance of blank}) \times 100$$

Statistical Analysis

Data were analysed using one-way analysis of variance (ANOVA), and mean differences were considered statistically significant at P < 0.05 based on Duncan's multiple range test. All statistical analyses were performed using SPSS software (Version 21.0; SPSS Inc., Chicago, IL, USA). Results are presented as mean of triplicate

determinations $\pm$ standard deviation (SD) of three independent replicates ($n = 3$). Duncan's Multiple Range Test was selected as the post hoc procedure due to its higher sensitivity in detecting treatment differences in agricultural experiments, where subtle variations among treatments are biologically significant. Compared to Tukey's HSD, Duncan's test provides greater discriminatory power while maintaining acceptable control of Type I error under controlled experimental conditions.

Result and Discussion

Physicochemical Properties of Water Samples

The physicochemical properties of water samples from rural and semi-urban regions are summarised in Table 1. Diluted sewage water values were compared with FAO irrigation water standards (Pescod, 1992). All samples exhibited pH within the recommended range (FAO, 1992; WHO, 1994). Electrical conductivity (0.98–1.93 mS/cm), TDS (630–1205 mg/L), TSS (115–240 mg/L), and TS (750–1420 mg/L) were below FAO thresholds, though semi-urban sewage water showed higher values than rural samples. Calcium hardness exceeded FAO limits in semi-urban sites (MASW: 360 mg/L; KKSU: 340 mg/L) but remained acceptable in rural sites. Nitrate ranged from 32–42 mg/L, with the highest in KNSW and the lowest in ETSW. BOD (11.8–22.4 mg/L) and COD (42–76 mg/L) were within FAO permissible limits, with higher values observed in semi-urban samples likely due to domestic and industrial pollutants (Alghobar and Suresha, 2017).

Potassium, sodium, and chloride showed no significant variation, while nitrogen registered the highest value in KNSW (42 mg/L) and the lowest value in the ETSW (32 mg/L) samples. Phosphate (2.8 mg/L), potassium (501 mg/L), and sulfate (42 mg/L) were maximal in the KKSU sample. ORP values were higher in rural well water (328–396 mV) than in semi-urban sewage water (279–280 mV), indicating greater oxidative capacity and microbial degradation potential in rural sources. Electrical conductivity and pH are key determinants of nutrient and contaminant mobility in the water-soil-plant-microbe continuum.

The reuse of domestic sewage wastewater provides a reliable water supply, enhances soil fertility, reduces dependence on freshwater and synthetic fertilisers, and can improve crop productivity when nutrient application is matched to crop needs (Chakrabarti, 1995; Khurana and Singh, 2012; Yang et al., 2015; Mishra et al., 2023; Heyde et al., 2025). Microbial analysis detected total and faecal coliforms in semi-urban wastewater, highlighting potential pathogen risks. However, on-farm biochar filtration could mitigate microbial contamination in sewage wastewater (Perez-Mercado et al., 2019).

Macro and micronutrient concentrations were generally within the permissible limits (Table 2); however, semi-urban samples showed slightly elevated Cr, Fe, and Zn exceeding FAO and WHO thresholds (Cr: 0.1 mg/L; Fe: 5 mg/L; Zn: 2 mg/L), which might be due to fertiliser and waste inputs. Long-term use of these wastewaters has not caused critical metal accumulation in soils, and studies have also reported improvements in soil structure and agricultural productivity with proper wastewater management (Lehrsch et al., 2008; Muyen et al., 2011). Overall, domestic wastewater, if managed effectively, represents a valuable resource for sustainable agriculture. The total coliform bacteria and faecal coliform bacteria were not detected in the well water irrigated rural areas, while the semi-urban area showed total coliform bacteria (MASW 130MPN/100ml) followed by KKSU (135 MPN/100ml). The faecal coliform bacteria in the analysed samples were found to be as follows: MASW (84 MPN/100ml) > KKSU (78MPN/100ml).

Table 1. Physicochemical Parameters of irrigation Water Samples

Parameters	ETSW	KNSW	MASW	KKSU	Permissible limit (WHO 1994)	Permissible limit (FAO, 1985)
pH	7.5	7.71	7.82	7.81	6.5-8.5	6.5-8.5
Electrical conductivity (mS/cm)	0.98	1.41	1.81	1.93	-	>3
Total solids (mg/L)	750	1015	1380	1420	-	-
Total dissolved solids (mg/L)	630	890	1140	1205	50-250	>2000
Total suspended solids (mg/L)	120	115	240	215	-	-
Mg hardness (mg/L)	34	37	51	53	-	-
Ca hardness (mg/L)	180	200	360	340	50-250	-
Total hardness (mg/L)	290	320	475	490	-	-
Total alkalinity (mg/L)	138	176	220	254	50-250	-
Chloride (mg/L)	98	140	268	288	-	1100
Nitrate(mg/L)	32	42	36	40	-	-
Phosphate (mg/L)	0.9	1.14	2.6	2.8	-	2
Sulphate (mg/L)	26	29	38	42	-	1000
Calcium (mg/L)	53	46	64	78	-	400
BOD (mg/L)	12	11.8	22.4	19.6	-	100
COD (mg/L)	46	42	76	70	-	80-500

DO (mg/L)	3.25	3.32	4.16	4.12		5.0
Oxidation and Reduction						
Potential (Eh) (mV)	398	326	279	280		300-500
Total Coliform (MPN/100ml)			130	135		
Faecal Coliform (MPN/100ml)			84	78		

Rural Areas: ETSW-Ettimadai sorghum-well water irrigation sample and KNSW- Kannamanaickanur sorghum well water irrigation sample. Semi-Urban Areas: MASW-Madukkarai sorghum-sewage wastewater irrigation sample and KKSWS-Kinathukadavu sorghum-sewage wastewater irrigation sample. Values are the mean of triplicate determinations of three independent samples (n=3).

Table 2. Macro-Micro elements and heavy metal composition of irrigation water samples (mg/L)

Element	ETSW	KNSW	MASW	KKSWS	Standard & Permissible Limit (FAO, 1985)
Be	0.1	0.2	0	0	-
Na	1687.64	472.34	1332.4	398.1	900
Mg	358.75	505.4	490.77	243.1	60
Al	12	4.06	0.129	4.64	-
K	393.1	480.33	267.4	501.0	0.2
Cr	0.5	0.65	0.091	0.76	0.1
Mn	0.1	0.09	0.015	0.1	0.2
Fe	4.9	4.82	8.154	3.4	5
Ni	0	0.18	0.025	0.7	5
Cu	0.08	0.058	0.029	0	0.1
Zn	0.2	2.63	0.178	3	2
As	0	0.76	0.149	3.5	-
Se	0	0	0	0.1	-
Mo	0.1	1.39	0.06	0	-
Cd	0	0	0	0	0.01
Pb	0	0	0	0	2
Ca*	53	46	64	78	400

Rural areas: ETSW-Ettimadai sorghum-well water irrigation sample and KNSW- Kannamanaickanur sorghum-well water irrigation sample. Semi-Urban areas: MASW-Madukkarai sorghum- sewage wastewater irrigation sample and KKSWS-Kinathukadavu sorghum-sewage wastewater irrigation sample, *Flame photometry Method.

Physicochemical Parameters of Soil Samples

The analysed soil parameters (Table 3) indicate enhanced soil fertility following irrigation with domestic sewage wastewater. Soil types, lime status, and texture were determined according to FAO standards (FAO, 1985). Soil pH ranged from 6.73 to 8.3, with a slight reduction observed in sewage water-irrigated soils. Electrical conductivity was elevated in the semi-urban sample MASS (0.46 dS/m). Total organic carbon (TOC) ranged from 0.79% to 2.3%, with the rural sample KNSS exhibiting the highest organic matter content.

Macronutrients (N, P, K) were significantly higher in the semi-urban wastewater-irrigated soil MASS (N: 238 kg/ha; P: 29 kg/ha; K: 904 kg/ha) compared to the rural ETSS soil, corroborating previous findings that sewage irrigation enhances soil nutrient levels (Khaskhoussy et al. 2013). Heavy metal concentrations were within permissible limits. While Fe varied between KNSS and KKSWS, Ni, As, Pb, and Cd were slightly higher in KKSWS but remained safe. Sorghum demonstrated moderate accumulation, suggesting partial exclusion mechanisms, whereas wheat exhibited natural resistance to Cr uptake (Akhtar et al., 2025). These results align with studies indicating minimal heavy metal accumulation risk under long-term wastewater irrigation (Dotaniya et al., 2018; Elfanssi et al., 2018).

Overall, domestic wastewater and well water irrigation improved soil physicochemical properties, including pH, TOC, and macronutrient content, without causing hazardous metal buildup. Regular application enhances soil fertility, promoting higher crop yields and improved nutritional quality, including elevated protein and antioxidant content (Agrawal et al., 2014; Roopika et al., 2024; Yang et al., 2015). These findings underscore the potential of properly managed domestic wastewater as a sustainable resource for agricultural intensification, provided that periodic monitoring ensures long-term soil and crop safety.

Rural Areas: ETSS - Ettimadai sorghum-well water irrigation soil sample and KNSS- Kannamanaickanur sorghum-well water irrigation soil sample. Semi-Urban Areas: MASS- Madukkarai sorghum-sewage wastewater irrigation soil sample and KKSWS- Kinathukadavu sorghum-sewage wastewater irrigation soil sample. Soil parameters (N, P, K and TOC) presented in Table 3 is expressed on a dry weight basis.

Table 3. Physicochemical Parameters of Soil Samples

Parameters	ETSS	KNSS	MASS	KKSS
Lime status	Calcareous	Non-calcareous	Calcareous	Non-calcareous
Soil texture	Clay loam	Sandy clay loam	Clay loam	sandy clay loam
Soil Type	Brown	Reddish Brown	Reddish Brown	Red
pH	8.3	6.73	7.98	7.20
EC (Ds/m)	0.25	0.15	0.46	0.30
TOC (%)	2.10	2.30	0.79	0.83
N (kg/ha)	202	199	238	176
P (kg/ha)	10	20	29	21
K (kg/ha)	499	471	904	540

Mineral Composition of Sorghum bicolor Grain Samples

The macro- and microelement contents, as well as the heavy metal contents of raw and boiled *S. bicolor* grains irrigated with domestic sewage wastewater (semi-urban) and well water (rural) are presented in Table 4. Sewage wastewater irrigation altered soil physicochemical properties, which affected nutrient and metal uptake by crops.

Sorghum grains from semi-urban wastewater-irrigated sites showed elevated concentrations of macroelements—Na, P, K, Ca, and Mg—while microelements such as Fe and Zn also increased. These increases were associated with nutrient-rich irrigation water, soil properties, and plant uptake efficiency, consistent with previous reports linking treated effluent irrigation to enhanced nutrient content in crops. Enhanced N and P availability under wastewater irrigation was associated with improved plant physiological traits, including plant height, leaf area, dry matter production, and grain yield. Among the nutritional parameters, total sugar content showed notable improvement. Heavy metal concentrations in Sorghum grains remained below permissible limits, indicating that properly managed domestic wastewater provides a safe, nutrient-rich alternative for irrigation, supporting both crop productivity and food safety (Daneshvar et al., 2020).

Table 4. Macro-Micro Elements and Heavy Metal Profile of *Sorghum bicolor* Whole Raw and Boiled Grain Samples (mg/kg)

Minerals	ETSR	ETSB	KNSR	KNSB	MASR	MASB	KKSR	KKSB	Permissible limit in Cereals (mg/kg) (Uddin et al., 2021)
Be	0	0	0.01	0.01	0	0	0.085	0	-
Na	244.51	633.55	163.13	1307.5	458.17	616.06	962.04	433.38	-
Mg	10.665	13.51	15.61	17.87	15.395	12.175	20.395	21.795	-
Al	36.405	32.375	34.87	26.65	10.485	56.12	18.685	7.84	-
K	426.66	179.88	414.39	358.22	732.5	194.95	236.54	125.84	-
Cr	1.16	0.91	1.065	1.135	0.84	0.61	1.175	0.025	1.3
Mn	7.035	3.785	5.115	5.065	3.09	3.6	5.625	0.21	-
Fe	12.05	21.785	28.84	34.885	48.565	51.015	41.025	47.06	-
Ni	0.065	0.035	0.06	0.01	0.01	0.035	0.035	0.015	-
Cu	0.215	0.175	0.235	0.16	0.14	0.11	0.225	0.20	20.0
Zn	1.335	2.075	1.815	0.715	0.54	0.94	0.915	0.01	60.0
As	0.01	0	0.025	0.01	0.01	0	0.045	0	0.1-0.2
Se	0.01	0	0.01	0	0	0	0	0	-
Mo	0.075	0.04	0.21	0.05	0.035	0.085	1.215	0.035	-
Cd	0	0	0	0	0	0	0	0	0.05-0.4
Pb	0.05	0.04	0.035	0.1	0.025	0.025	0.045	0	0.05-0.3
Ca*	13.5	13.9	14.3	12.8	13.8	14.1	15.11	14.9	-

Rural areas: ETSR-Ettimadai well water irrigation sorghum raw sample, ETSB-Ettimadai well water irrigation sorghum boiled sample, KNSR-Kannamanaickanur well water irrigation sorghum raw sample and KNSB-Kannamanaickanur well water irrigation sorghum boiled sample. Semi urban areas: MASR-Madukkarai sewage wastewater irrigation sorghum raw sample, MASB-Madukkarai sewage wastewater irrigation sorghum boiled sample, KKSR-Kinathukadavu sewage wastewater irrigation sorghum raw sample and KKSB-Kinathukadavu sewage wastewater irrigation sorghum boiled sample, *Flame Photometry Method.

Heavy Metal Composition

Heavy metal levels in *Sorghum bicolor* grains irrigated with domestic sewage wastewater (semi-urban, KKSR) and well water (rural) are summarised in Table 4. Elevated concentrations of certain metals were observed in semi-

urban wastewater-irrigated grains. Chromium (Cr) ranged from 0.025 to 1.175 mg/kg, slightly exceeding the WHO/EU (1983) limit of 0.1 mg/kg. Iron (Fe) varied from 12.05 to 51.06 mg/kg, with the boiled MASB sample surpassing the permissible limit of 48 mg/kg. Zinc (Zn) levels (0.01–2.075 mg/kg) in semi-urban samples exceeded the recommended threshold of 0.3 mg/kg, whereas copper (Cu: 0.11–0.225 mg/kg), nickel (Ni: 0.01–0.065 mg/kg), and arsenic (As: 0–0.56 mg/kg) remained within safe limits. Lead (Pb) reached up to 0.045 mg/kg, slightly above the safe limit of 0.03 mg/kg, likely due to localised upstream waste inputs. Manganese (Mn: 0.21–6.03 mg/kg) exceeded the WHO threshold of 0.2 mg/kg, while cadmium (Cd) was not detected.

These results align with previous studies showing that crops irrigated with treated wastewater may accumulate certain heavy metals, but levels generally remain within FAO/WHO safety limits (Salakinkop and Hunshal, 2014; Adam et al., 2022; Mbarki et al., 2022). *Sorghum bicolor* exhibits tolerance to heavy metals and can uptake them without compromising the safety of edible grains when irrigation involves properly treated domestic wastewater. Controlled use of such wastewater improves soil fertility and plant growth and reduces reliance on chemical fertilisers, while minimising health risks (Khan, 2018). Continuous monitoring is recommended to ensure safe heavy metal levels in food crops.

Proximate Composition

The proximate composition of *Sorghum bicolor* grains from semi-urban and rural sites is presented in Table 5, showing statistically significant differences ($p < 0.05$). Moisture content of the grain samples ranged from 6.88% (KKSBB) to 11.75% (ETSR). Ash content (1.37–5.72 g/100 g) aligned with values reported by David et al. (2014), indicating variation in mineral availability due to irrigation sources. Lipid content ranged from 6.37 (KKSBB) to 12.83 g/100 g (ETSB), while crude protein ranged from 9.03 to 16.44 g/100 g, with the highest in KNSR and lowest in MASR, comparable to those of USDA-ARS (2015) values but lower than those values reported by Cadogan and Finn (2010). Crude fibre was highest in ETSB (1.59 g/100 g), and total crude carbohydrates or Nitrogen-Free Extractives (NFE) varied, with KKSBB showing the highest value of 76.23 g/100 g. Among the experimental samples, the highest gross energy content was in ETSR (1906.13 kJ/100 g), significantly. Overall, rural and semi-urban samples were nutritionally comparable, supporting their equal suitability for consumption.

Processing (boiling) influenced proximate composition: moisture, lipids, NFE, and energy content generally decreased, while effects on protein, ash, and fibre were variable. Increased ash in boiled wastewater-irrigated samples suggests mineral concentration or enhanced uptake, whereas fibre often increased due to the relative concentration of structural polysaccharides and non-starch polysaccharides. Carbohydrate losses during boiling led to lower NFE. These findings indicate that wastewater irrigation enhances mineral content, but its impact on other nutrients depends on soil properties, water quality, and genotype (Tanwar et al., 2023; Adebo and Kesa, 2023). Previous studies also show that domestic wastewater irrigation improves crop productivity, sustainability, and nutrient content, with cyclic irrigation of well water and treated sewage water further enhancing biomass and yield (Salakinkop and Hunshal, 2014; Minhas et al., 2015; Soni et al., 2015).

Table 5. Proximate composition of *Sorghum bicolor* raw and boiled whole grain samples

Samples	Moisture (%)	Crude Ash (g/100g)	Crude Lipid (g/100g)	Crude Protein (g/100g)	Crude Fibre (g/100g)	NFE (g/100g)	Energy (kJ/100g)
ETSR	11.75 ^a ±1.480	1.386 ^b ±0.510	12.83 ^a ±0.274	10.740 ^b ±0.535	0.619 ^b ±0.039	72.983 ^c ±0.729	1906.13 ^a ±13.79
ETSB	7.82 ^{cd} ±0.665	4.080 ^a ±0.238	8.069 ^c ±0.380	11.159 ^b ±0.920	1.590 ^a ±0.336	68.697 ^d ±1.704	1654.53 ^e ±16.98
KNSR	9.82 ^b ±0.803	1.372 ^b ±0.066	8.825 ^c ±0.282	14.940 ^a ±0.404	0.548 ^b ±0.041	70.198 ^d ±0.474	1779.94 ^{bc} ±6.232
KNSB	7.99 ^{cd} ±0.548	3.969 ^a ±0.082	8.743 ^c ±0.195	8.873 ^c ±0.439	0.440 ^b ±0.144	77.204 ^a ±0.239	1779.98 ^{bc} ±2.397
MASR	9.63 ^{bc} ±0.544	3.954 ^a ±0.043	11.69 ^{ab} ±1.631	8.172 ^c ±0.304	0.560 ^b ±0.038	77.322 ^a ±1.964	1883.23 ^a ±34.32
MASB	8.88 ^{bc} ±0.440	5.720 ^a ±0.404	6.516 ^d ±0.996	11.90 ^b ±0.660	0.590 ^b ±0.328	75.731 ^{ab} ±1.218	1728.63 ^d ±27.03
KKSRR	9.52 ^{bc} ±0.443	2.630 ^b ±0.523	10.53 ^b ±0.595	9.570 ^{bc} ±1.610	0.530 ^b ±0.301	74.250 ^{bc} ±1.442	1813.80 ^b ±53.48
KKSBB	6.88 ^d ±0.120	1.820 ^c ±0.104	6.370 ^d ±0.972	13.30 ^a ±0.350	1.270 ^b ±0.502	76.230 ^a ±1.254	1752.19 ^{bd} ±11.48

ETSR-Ettimadai (Rural) well water irrigated sorghum raw grains, ETSB- Ettimadai (Rural) well water irrigated sorghum boiled grains, KNSR-Kannamanaickanur (Rural) well water irrigated sorghum raw grains , KNSB-Kannamanaickanur (Rural) well water irrigated sorghum boiled grains, MASR- Madukkarai (Semi urban) sewage wastewater irrigated sorghum raw grains, MASB- Madukkarai (Semi urban) sewage wastewater irrigated sorghum raw grains, KKSRR-Kinathukadavu (Semi urban) sewage waste water irrigated sorghum raw grains, KKSBB-Kinathukadavu (Semi urban) sewage wastewater irrigated sorghum boiled grains. Each value is expressed as the mean of triplicate determinations; ± standard deviation (n=3). Mean values with different letters in a column are significantly different ($p < 0.05$).

Extract Yield Percentage, Total Phenolics and Tannin Content of *Sorghum bicolor* Grain Samples

The results of the extract yield percentage, total phenolics, tannins and flavonoids concentration of *Sorghum bicolor* grain samples collected from semi-urban and rural areas under varying irrigation practices are given in Table 6. The

extract yields ranged from 10% to 16%, with the highest yield recorded in raw rural sorghum (KNSR, 16%) and the lowest in both rural and semi-urban processed samples (ETSB and MASB, 10%).

Sorghum is recognised as a rich source of bioactive compounds, including 3-deoxyanthocyanidins, tannins, tocopherols (vitamin E), carotenoids, and various antioxidants (Minhas et al., 2015). Total phenolic content, a key indicator of antioxidant capacity, ranged from 158.66 to 206.96 mg tannic acid equivalents (TAE)/g extract. Notably, processed samples exhibited higher phenolic concentrations than their raw counterparts, with the maximum observed in boiled rural sorghum (KNSB, 206.96 mg TAE/g) and the minimum in the semi-urban processed sample (MASB, 158.66 mg TAE/g).

Tannin content followed a similar trend, with the highest level detected in boiled rural sorghum (KNSB, 125.33 mg TAE/g), while the lowest was recorded in MASB (99.11 mg TAE/g) (Table 7). Flavonoids in sorghum are predominantly localised in the outer grain layers and contribute to its antioxidant potential, anti-inflammatory activity, and other health-promoting effects (de Moraes Cardoso et al., 2015). Furthermore, pigmented sorghum varieties have been reported to possess lower levels of rapidly digestible starch and generate higher resistant starch contents, which are associated with beneficial effects on glycemic response, particularly in type 2 diabetics in humans (Wu et al., 2017). Recent studies have also highlighted the potential of sorghum tannin–gluten interactions in developing functional food matrices aimed at reducing starch digestibility (Rocchetti et al., 2020). In addition, 3-deoxyanthocyanidin pigments from sorghum are emerging as promising natural food colourants due to their exceptional stability during food processing (Girard and Awika, 2020).

Table 6. Extract yield, total phenolics, tannins and flavonoids content of *Sorghum bicolor* experimental grain samples

Samples	Extract Yield (%)	Total Phenolics**	Total Tannins**	Total Flavonoids*
ETSR	13	181.77 ^e ±1.33	107.55 ^d ±0.44	26.83 ^d ±0.75
ETSB	10	206.22 ^{ab} ±2.47	120.29 ^{abc} ±0.51	26.5 ^d ±0.88
KNSR	16	198.96 ^c ±2.44	120.88 ^{abc} ±2.7	39.16 ^b ±0.66
KNSB	12	206.96 ^a ±1.35	124.29 ^a ±1.79	44.66 ^a ±1.89
MASR	14	190.66 ^d ±4.00	116.44 ^c ±3.11	35.61 ^c ±0.71
MASB	10	158.66 ^f ±0.88	99.11 ^e ±1.33	17.44 ^e ±0.83
KKSR	13	202.22 ^{bc} ±1.60	119.25 ^{bc} ±2.95	36.77 ^c ±0.47
KKSB	12	204.44 ^{ab} ±3.79	121.48 ^{ab} ±4.75	25.69 ^d ±0.85

Rural Areas: ETSR-Ettimadai Sorghum –well water irrigated raw sample, ETSB-Ettimadai Sorghum well water irrigated boiled sample, KNSR-Kannamanaickanur sorghum-well water irrigated raw sample and KNSB-Kannamanaickanur sorghum–well water irrigated boiled sample. Semi Urban Areas: MASR-Madukkarai sorghum sewage wastewater irrigated raw sample, MASB-Madukkarai sorghum-sewage wastewater irrigated boiled sample, KKSR-Kinathukadavu sorghum-sewage wastewater irrigated raw sample and KKSB- Kinathukadavu sorghum–sewage wastewater irrigated boiled sample. Each value is expressed as the mean of triplicate determinations; ± standard deviation (n=3). Mean values with different letters in a column are significantly different (p<0.05). **mg of Tannic acid equivalent (TAE)/g extract; *mg of Rutin equivalents (RUE)/g extract.

Total Flavonoids Content

Flavonoids, a major subclass of phenolic compounds, are associated with multiple health benefits, including reduced risk of colorectal, pancreatic, and breast cancers (de Moraes Cardoso et al. 2015). The total flavonoid content (TFC) of *Sorghum bicolor* grains, expressed as Rutin equivalents (RUE)/g extract (Table 6), ranged from 17.44 to 44.66 mg RUE/g extract (Table 6). Boiled rural sorghum (KNSB) exhibited the highest TFC (44.66 mg RUE/g), while the semi-urban processed sample (MASB) showed the lowest (17.44 mg RUE/g) (Table 6).

Sorghum bicolor flavonoids are predominantly localised in the outer grain layers and strongly influenced by genetic factors, contributing to the plant's defense against environmental stressors (de Moraes Cardoso et al., 2015; Xiong et al., 2019). Proanthocyanidins, in particular, are implicated in glycemic regulation and anticancer activity via mechanisms such as α-amylase, α-glycosidase and aromatase inhibition, highlighting sorghum's potential as a functional food.

Both rural and semi-urban (sewage wastewater–irrigated) samples have registered elevated levels of total phenolics, tannins, and flavonoids, underscoring their nutritional value and supporting the sustainable reuse of domestic wastewater for agriculture. Additionally, sorghum tannin–gluten complexes show promising applications in edible films, biomedical materials, adhesives and biodegradable plastics (Rocchetti et al., 2020).

Antioxidant Activities of *Sorghum bicolor*

Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant potential of *Sorghum bicolor* whole grains was markedly higher than that of commonly consumed cereals such as rice, wheat, and oats, underscoring sorghum as a rich source of natural antioxidants (de Morais Cardoso et al., 2015). The Ferric Reducing Antioxidant Power (FRAP) assay revealed significant differences ($P < 0.05$) among raw and processed sorghum extracts, with values ranging from 10,673.80 to 12,215.56 mmol Fe(II)/g extract (Table 7). The highest FRAP activity was observed in raw semi-urban sorghum (MASR; 12,215.56 mmol Fe(II)/g), while the lowest occurred in boiled semi-urban sorghum (KKSBB; 10,673.80 mmol Fe(II)/g).

The elevated antioxidant capacity is primarily attributed to phenolic compounds, including 3-deoxyanthocyanidins, high molecular weight condensed tannins, flavonols, and kafirins, which contain hydroxyl (-OH) functional groups (Hagerman et al., 1998). These bioactives are distributed throughout the sorghum grain and contribute not only to its ferric-reducing power but also to chemopreventive effects, such as anti-cancer activity against human colon cancer cells (Zhu, 2019).

Table 7. Effect of *Sorghum bicolor* raw and boiled grain sample extracts on the antioxidant activities

Samples	FRAP ^a (mmol Fe(II)/g extract)	Metal Chelating ^b (mg EDTA/g extract)	(TEAC mmol/g extract)	
			DPPH ^c	ABTS ^d
ETSR	11991.91 ^b ±92.88	4.94 ^d ±0.106	2423.58 ^e ±15.64	17727.49 ^c ±132.4
ETSB	10807.04 ^e ±92.88	3.55 ^g ±0.080	2717.85 ^g ±12.94	17605.83 ^c ±95.72
KNSR	11387.58 ^d ±111.49	6.11 ^a ±0.102	2543.7 ^d ±9.49	20155.71 ^a ±87.99
KNSB	10602.42 ^e ±79.482	5.42 ^c ±0.085	2605.94 ^c ±15.64	19819.9b±94.98
MASR	12215.56 ^a ±79.48	5.59 ^b ±0.103	1681.69 ^f ±16.44	14603.40 ^d ±36.73
MASB	11644.53 ^c ±79.48	3.82 ^f ±0.06	2651.53 ^b ±34.6	13362.53 ^e ±107.6
KKSRR	11725.43 ^c ±57.69	4.81 ^d ±0.07	2396.64 ^e ±27.09	13464.72 ^e ±89.19
KKSBB	10673.80 ^{ef} ±57.10	8.04 ^e ±0.051	2643.24 ^b ±19.98	17605.83 ^c ±95.72
BHA	350760.45±72476.70	10.48±0.060	655137.0±61415.86	386368.04±9622.0
RUTIN	174032.83±26869.46	-	433569.06±23178.33	-
Tannic Acid	637663.57±55147.36	-	751735.57±62890.8	-
Trolox	562955.032±42130.91	-	-	-
Ascorbic Acid	730073.75±89814.82	-	599419.74±104323.	493310.06±1125.9

^aConcentration of substance having ferric TPTZ reducing ability expressed as mmol Fe (II) equivalents.

^bConcentration of substance having metal chelating ability expressed as mg EDTA Equivalents per g.

^cTrolox equivalent antioxidant capacity (TEAC), (mmol equivalent Trolox performed by using DPPH·)

^dTrolox equivalent antioxidant capacity (TEAC), (mmol equivalent Trolox performed by using ABTS·⁺).

Each value is expressed as the mean of triplicate determinations ± standard deviation (n=3). Mean values with different letters in a column are significantly different ($p < 0.05$). ETSR-Ettimadai Sorghum–well water irrigated raw sample, ETSB-Ettimadai Sorghum well water irrigated boiled sample, KNSR-Kannamanaickanur Sorghum-well water irrigated raw sample and KNSB- Kannamanaickanur Sorghum-well water irrigated boiled sample (Rural areas). MASR- Madukkarai Sorghum-sewage wastewater irrigated raw sample, MASB-Madukkarai Sorghum-sewage wastewater irrigated boiled sample, KKSRR-Kinathukadavu Sorghum-sewage wastewater irrigated raw sample and KKSBB-Kinathukadavu Sorghum-sewage wastewater irrigated boiled sample (semi-urban areas).

Metal Chelating Activity

Metal-mediated catalysed oxidation, particularly Fenton-type reactions, contributes to lipid peroxidation, as reduced iron (Fe²⁺) reacts with lipid hydroperoxides (ROOH) to form alkoxy and peroxy radicals. Ferrous and cupric ions, due to their catalytic role in oxidative reactions, are effectively targeted by phenolic compounds, which inhibit metal-catalysed oxidation through chelation (Vanamala et al., 2018; Nithiyanantham et al., 2012; Bhattacharjee, 2014).

In the present study, the highest ferrous ion-chelating activity was observed in boiled semi-urban sorghum (KKSBB; 8.04 mg EDTA/g extract), whereas the lowest activity was recorded in boiled rural sorghum (ETSB; 3.55 mg EDTA/g extract) (Table 7). Although these values were lower than those of the synthetic antioxidant butylated hydroxyanisole (BHA; 10.59 mg EDTA/g extract), the chelating capacity of both raw and processed sorghum extracts remains notable. Thermal processing, including boiling or extrusion, enhanced metal chelation across all cultivars, likely due to the formation of novel antioxidant compounds such as melanoidins (Kumari et al., 2017).

DPPH· Scavenging Activity

The DPPH· assay evaluates the free radical-scavenging ability of antioxidants by measuring the reduction of the stable DPPH radical (absorbance at 517 nm) to 1, 1-diphenyl-2-picrylhydrazine, with the extent of decolourisation reflecting antioxidant capacity.

Sorghum bicolor extracts showed significant variation in DPPH scavenging between raw and hydrothermally processed samples (Table 7). The highest activity was recorded in raw rural sorghum seeds (KNSB; 20,155.71 mmol TEAC/g extract), while the lowest occurred in raw semi-urban sorghum (KKSr; 13,464.72 mmol TEAC/g extract). Overall, DPPH values were lower than ABTS^{•+} activities, likely due to differences in radical types and phenolic composition. These findings highlight the strong antioxidant potential of *Sorghum bicolor*, confirming its value as a functional food and natural source of dietary antioxidants.

Antioxidant Activity by the ABTS^{•+} Assay

Phenolic compounds in sorghum protect plants against pest-related diseases and act as antioxidants, scavenging free radicals implicated in cancer, rheumatoid arthritis, cataracts, atherosclerosis, and inflammatory bowel disease (Rao et al., 2018; Punia et al., 2021). These compounds effectively neutralise ABTS^{•+} radicals via hydrogen atom donation, electron transfer, or a combination of both (Siddhuraju and Becker, 2007).

ABTS^{•+} scavenging activity of *Sorghum bicolor* (Table 7) showed significant differences ($P < 0.05$) among samples, with the highest activity in boiled rural sorghum (ETSB; 2717.85 mmol TEAC/g extract) and the lowest in raw semi-urban sorghum (MASR; 1681.69 mmol TEAC/g extract). These values are comparable to standard antioxidants such as BHA and α -tocopherol, underscoring the functional potential of sorghum extracts. High-molecular-weight phenolics enhance free-radical quenching, particularly against ABTS^{•+}, highlighting sorghum grains as promising functional foods and nutraceuticals. Similar trends have been reported in *Medicago sativa*, where irrigation with raw wastewater increased antioxidant enzyme activities and soluble metabolites without exceeding tolerance thresholds (Elfanssi et al., 2018).

Hydroxyl Radical Scavenging Activity

The hydroxyl radical ($\cdot\text{OH}$) is a highly reactive oxygen species, capable of indiscriminate

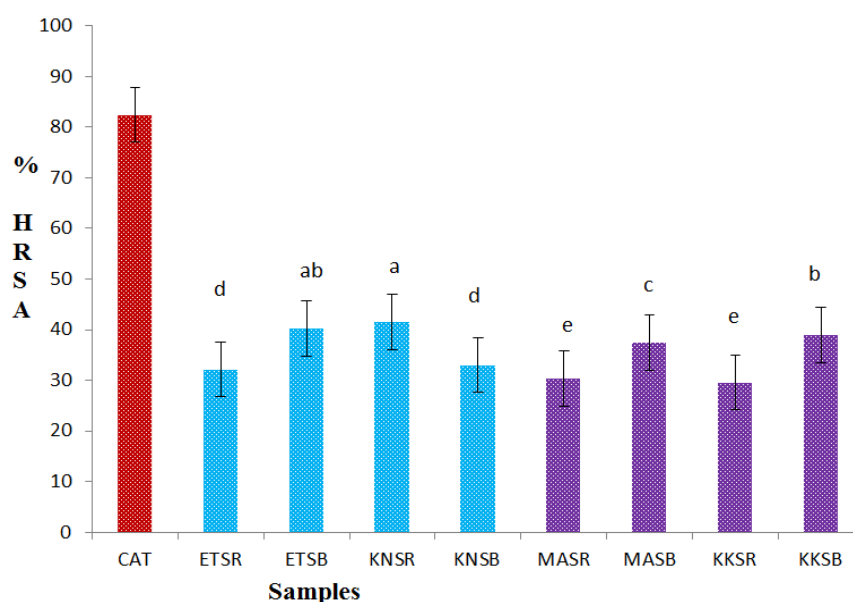


Fig. 1. Hydroxyl radical anion scavenging activity of raw and processed *S. bicolor* grain samples. ETSR-Ettimadai (Rural Area) Sorghum-well water irrigated raw, ETSB-Ettimadai (Rural Area) Sorghum-well water irrigated boiled, KNSR-Kannamanaickanur (Rural Area) Sorghum-well water irrigated raw, KNSB-Kannamanaickanur (Rural Area) Sorghum-well water irrigated boiled, MASR-Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated raw, MASB-Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated boiled, KKSr-Kinathukadavu (Semi-Urban) Sorghum-sewage wastewater irrigated raw, KKSb-Kinathukadavu (Semi-Urban) Sorghum-sewage wastewater irrigated boiled. Values are the mean of triplicate determinations $\pm$ standard deviations ($n = 3$).

Mean values followed by different superscript letters in the same bars are significantly ($p < 0.05$) different interactions with lipids, proteins, carbohydrates, and nucleic acids, leading to mutagenesis, carcinogenesis, and cytotoxicity. Its reaction with DNA can induce strand breaks, while interaction with aromatic compounds forms hydroxycyclohexadienyl radicals that may further react with oxygen to generate peroxy radicals or dehydrate into phenoxy-type radicals (Loganayaki et al., 2013). In this study, all analyzed *Sorghum bicolor* samples exhibited significant hydroxyl radical scavenging activity, ranging from 29.54% to 41% (Figure 1). The highest activity was observed in raw rural sorghum (KNSR; 41%), and the lowest in raw semi-urban sorghum seeds (KKSr; 29.54%), with the overall trend: KNSR > ETSB > KKSb > MASB > KNSB > ETSR > MASR > KKSr. Neutralisation of $\cdot\text{OH}$ is a

critical antioxidant defence, protecting cellular components from oxidative damage. The observed scavenging activity, coupled with high phenolic content, underscores the potential of sorghum as a functional food with enhanced nutritional and health-promoting properties (Punia et al., 2021).

Superoxide Anion and Nitric Oxide Radicals Scavenging Activity

Free radicals, including superoxide anions ($O_2^{\cdot-}$) and nitric oxide (NO), are key intermediates in biological metabolism. Overproduction of these species leads to oxidative stress, disrupting the balance between pro-oxidants and antioxidants and contributing to chronic disease and pathogenesis. *Sorghum bicolor* extracts exhibit potent antioxidant activity, largely due to their high polyphenol content (Siddhuraju and Becker, 2007).

Superoxide anions, generated mainly by the mitochondrial electron transport chain and enzymes such as lipoxygenase, peroxidase, NADPH oxidase, and xanthine oxidase, can damage cellular components and DNA, promoting disease progression (Nithiyanantham et al., 2013; Devi et al., 2012). In this study, all sorghum samples showed significant superoxide anion scavenging activity, ranging from 44% to 93%, with thermal processing enhancing the activity. The highest activity was observed in boiled rural sorghum (ETSB; 93.55%), exceeding common antioxidants such as BHA (69%), Trolox (49%), and rutin (76%), and comparable to catechin (94%). The lowest was observed in semi-urban processed sorghum (MASB; 75.37%) (Figure 2).

Nitric oxide, although physiologically important, can induce inflammation and cancer when in excess. NO generated from sodium nitroprusside (SNP) in phosphate-buffered saline reacts with oxygen to form nitrites, which are quantified using the Griess reaction (Apea-Bah et al., 2016). Both raw and processed sorghum samples demonstrated notable NO scavenging activity, with the highest in raw semi-urban sorghum (KKSR; 75%)—surpassing ascorbic acid (57.9%) and quercetin (62.1%)—and the lowest in boiled rural sorghum (KNSB; 62%) (Figure 3). This activity is likely due to the antioxidant compounds' capacity to bind and neutralise NO, preventing its conversion to reactive nitrogen species such as nitrites and nitrates.

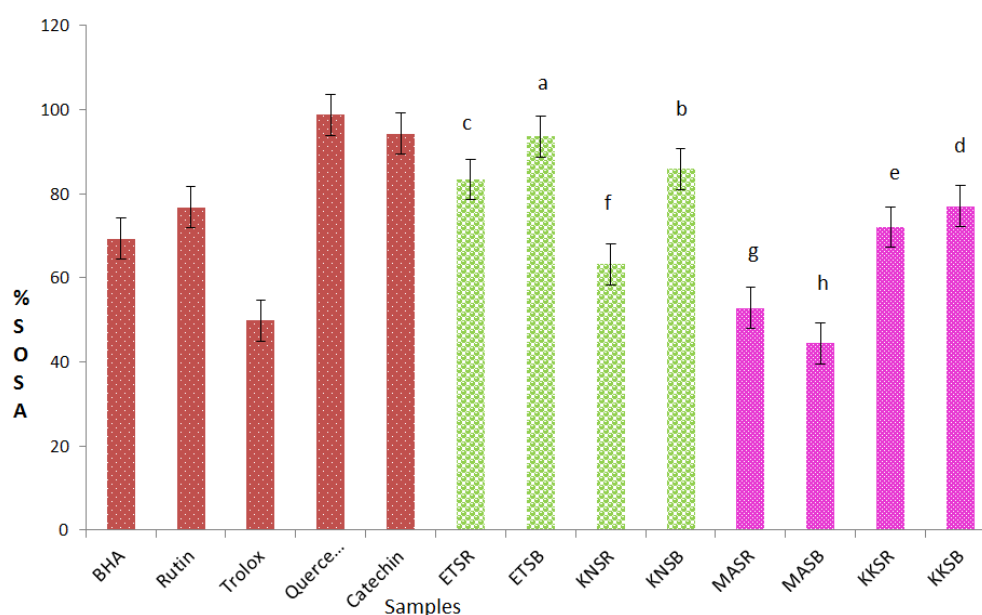


Fig. 2. Superoxide anion radical scavenging activity of raw and processed *Sorghum bicolor* experimental grain samples

ETSR-Ettimadai (Rural Area) Sorghum-well water irrigated raw, ETSB-Ettimadai (Rural Area) Sorghum-well water irrigated boiled, KNSR-Kannamanaickanur (Rural Area) Sorghum-well water irrigated raw, KNSB-Kannamanaickanur (Rural Area) Sorghum-well water irrigated boiled, MASR-Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated raw, MASB-Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated raw, KKSR-Kinathukadavu (Semi-Urban) Sorghum-sewage wastewater irrigated raw, KKSb-Kinathukadavu (Semi-Urban) Sorghum-sewage wastewater irrigated boiled. Each value is expressed as a mean of triplicate determinations $\pm$ standard deviation ($n=3$). Mean values followed by different superscript letters in the same bars are significantly different ($p < 0.05$).

ETSR– Ettimadai (Rural Area) Sorghum – well water irrigated raw, ETSB- Ettimadai (Rural Area) Sorghum-well water irrigated boiled, KNSR – Kannamanaickanur (Rural Area) Sorghum well water irrigated raw, KNSB – Kannamanaickanur (Rural Area) Sorghum well water irrigated boiled, MASR-Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated raw, MASB- Madukkarai (Semi-Urban) Sorghum-sewage wastewater irrigated raw, KKSR-Kinathukadavu (Semi-Urban) Sorghum-sewage wastewater irrigated raw, KKSb- Kinathukadavu (Semi-

Urban) Sorghum-sewage wastewater irrigated boiled. Values are the mean of triplicate determinations $\pm$ standard deviations ($n=3$). Mean values followed by different superscript letters in the same bars are significantly ($p<0.05$) different.

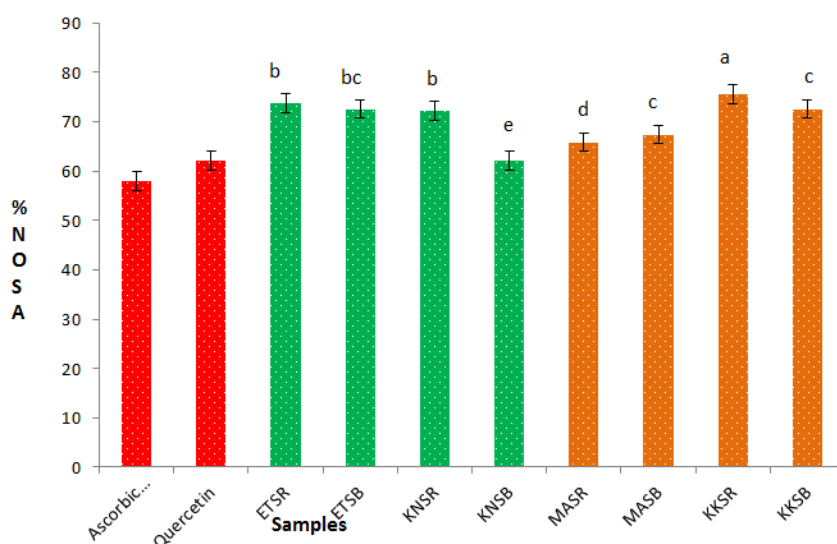


Fig. 3. Nitric oxide radical scavenging of raw and processed *Sorghum bicolor* grain samples

Conclusion

This study comprehensively assesses the effects of domestic sewage wastewater and well water irrigation on soil physicochemical properties and the nutritional quality of post-harvest *Sorghum bicolor* grains across different agroecological fields in Coimbatore, Tamil Nadu. The findings show that irrigation with treated domestic sewage wastewater significantly improves soil fertility by increasing the availability of key macronutrients, nitrogen (N), phosphorus (P), potassium (K), and organic carbon. These improvements in soil nutrient status were reflected in higher crop yields and enhanced nutritional profiles of sorghum grains, particularly with increased protein, carbohydrate, and energy content when compared to those irrigated with conventional well water. While the concentrations of heavy metals in this study remained within permissible thresholds, the findings emphasise the necessity for continuous monitoring, stringent regulatory oversight and adherence to safety standards to mitigate long-term risks. Therefore, the results highlight the potential of treated (diluted) domestic sewage wastewater as a viable and sustainable alternative for irrigation in water-stressed regions. When managed properly, this practice not only enhances agricultural productivity and food security but also contributes to environmental sustainability by reducing the discharge of untreated domestic wastewater into aquatic ecosystems and decreasing dependence on synthetic fertilisers. Future investigations should focus on periodic and long-term monitoring of heavy metal dynamics, crop rotation, crop biomass production, microbial contamination risks and localised/ traditional knowledge-based guidelines to optimise benefits while ensuring soil health, crop and food safety and agroecological integrity.

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Author Contributions

Dhanya V conducted field investigations; curated data; performed laboratory analyses; carried out formal data analysis; drafted the original manuscript; and prepared visualisations and figures. **Siddhuraju P** led concept formation; supervised the project; enhanced the methodology; reviewed and edited the manuscript; validated the results; prepared visualisations; and approved the final version.

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Data availability

The data and material that support the findings of this study are available from the corresponding author, upon request. All sources used are properly disclosed in the manuscript.

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Not applicable.

Competing interest

The authors declare no competing interests.

Ethics approval

Not applicable.



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