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Comparative Evaluation of Domestic Sewage and Well Water Irrigation on the Mineral Profile, Nutritional, and Nutraceutical Attributes of *Cajanus cajan* (L.) Millsp.

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Abstract— *Water scarcity has severely impacted the global economy, livelihoods, and environmental quality, driving the use of municipal and industrial wastewater as an alternative irrigation source in urban and peri-urban agriculture. This practice addresses water deficits but raises concerns about risks to human and environmental health from contaminants. This study evaluated the effect of diluted domestic sewage wastewater (used in semi-urban Palakkad) versus well water (used in rural areas) on pigeon pea (Cajanus cajan). We conducted a comprehensive analysis of irrigation water, soil, and various plant parts (seeds, leaves, stems, pods) for physicochemical parameters, proximate composition, full mineral profile, heavy metals, and in vitro antioxidant activity. Results indicated that most physicochemical parameters of the irrigation water were within FAO permissible limits, though the semi-urban source showed elevated iron, phosphate, and alkalinity. Critically, concentrations of toxic heavy metals (Ni, Cr, Cd, Pb, Cu) in soils and, most importantly, in the edible seeds remained well below WHO/FAO safety thresholds. Proximate analysis confirmed good nutritional quality in seeds from both sources (e.g., protein: 15.5-17.6 g/100g). Plants irrigated with both water types exhibited significant in vitro antioxidant activity across five complementary assays (FRAP, DPPH, ABTS, Metal Chelating, NO Scavenging), which was strongly correlated with phenolic content. A notable finding was the elevated iron content in seeds, suggesting a natural bioaccumulation propensity in C. cajan. The study concludes that, under the observed conditions, the use of diluted domestic sewage wastewater did not induce harmful heavy metal accumulation in C. cajan, maintained its nutritional value, and preserved its bioactive potential. This supports its role as a viable and sustainable irrigation alternative, contributing to water security and nutrient recycling. Non-seed tissues (leaves, pods) showed high phenolic content, indicating value for nutraceutical use or animal feed.*

Keywords—* *Cajanus cajan, sewage wastewater irrigation, heavy metals, food safety, proximate composition, antioxidant activity, sustainable agriculture.

I. INTRODUCTION

The global population is expected to exceed nine billion people by 2050. Population increases are expected to further increase water usage and wastewater production. The world is facing a water quality crisis resulting from continuous population growth, urbanization, land use change, industrialization, food production practices, increased living standards, unsustainable water use practices and wastewater management strategies. Freshwater resources are continuously depleting over time due to a combination of climatic, political, and anthropogenic factors. This growing scarcity has compelled many farmers to use sewage water as an alternative irrigation source for cultivation. Wastewater has a direct impact on the biological diversity of aquatic ecosystems and its inappropriate management is capable of disrupting the fundamental integrity of life support systems, on which a wide range of sectors, from urban development to food production and industry (Buonocorea et al., 2016; Khan et al., 2025).

Use of domestic and industrial wastewater in agriculture for irrigating crops appears to be a lucrative option. Besides being a source of irrigation water, these waste waters contain appreciable amounts of plant nutrients. In India, total wastewater generated per annum from 200 cities is about 2600 Mm3 and also the use of sewage effluents for irrigating agricultural lands is on the rise, especially in the peri-urban area. The increasing demand for freshwater, driven by rapid urbanization and residential development, has intensified pressure on existing water resources. Consequently, the use of sewage water for irrigation in agricultural practices has become increasingly common. This trend substantially alters the physicochemical characteristics of natural water bodies, generates significant economic activity, and supports numerous livelihoods, particularly among socioeconomically disadvantaged farming communities. However, the reuse of wastewater in agriculture presents several environmental and health-related risks. Some impacts are short-term, such as contamination by microbial pathogens, while others are long-term, including the gradual accumulation of salts and heavy metals in the soil, which can adversely affect soil fertility and crop productivity. The controlled reuse of treated wastewater offers a potential strategy to mitigate pressure on conventional freshwater resources by redirecting part of this water for agricultural and industrial applications. The nutrient content present in treated wastewater can enhance plant growth, thereby transforming potential pollutants into valuable resources. Given that the agricultural sector accounts for approximately 92% of global freshwater consumption, wastewater reuse represents a pragmatic approach toward sustainable water management. Nonetheless, in many regions, untreated sewage and industrial effluents are directly discharged onto agricultural lands and used for cultivating crops, including vegetables, posing considerable ecological and public health concerns (FAO, 2010; Liu et al., 2015; Balkhair and Ashraf, 2016; Shakir et al., 2017).

The utilization of wastewater treatment plant effluents for irrigation in agricultural and green areas offers several significant advantages, including cost-effectiveness, year-round availability, and reduced treatment and disposal expenses (Ganji et al., 2024). These effluents are often rich sources of organic matter and essential plant nutrients, which can enhance soil fertility and crop productivity. However, their continuous application can also lead to the accumulation of heavy metals such as Fe, Mn, Cu, Zn, Pb, Cr, Ni, Cd, and Co in the receiving soils. The elevated concentrations of these metals pose serious environmental and health concerns, as they may enter the food chain through the cultivation of edible crops. Consequently, the consumption of food grown using sewage wastewater irrigation increases the potential risk of heavy metal exposure to the general population. (Surdyk et al., 2025; Siddhuraju et al., 2025)

Pigeon pea (*Cajanus cajan*) is an important grain legume cultivated and consumed extensively across tropical and semi-arid regions of the world, including Asia, Africa, Latin America, and the Caribbean (Fatokimi andTanimonure, 2021). The crop exhibits strong adaptability to rain-fed conditions owing to its deep root system, heat tolerance, and rapid growth rate, which make it particularly suitable for cultivation in semi-arid environments. Globally, pigeon pea ranks as the sixth most important legume, occupying approximately 5.4 million hectares, with an estimated annual production of 4.49 million tons. It is commonly fried with spices, consumed as germinated seeds either raw or cooked, or combined with cereals to enhance nutritional value. Pigeon pea offers multiple agronomic and nutritional benefits, serving as both a valuable source of human food and animal feed while also contributing to soil fertility enhancement through nitrogen fixation. Nutritionally, pigeon pea seeds are rich in protein (20–22%), fat (1–2%), carbohydrates (approximately 65%), and ash (6.8%), and they provide considerable amounts of dietary fiber and essential minerals. Moreover, pigeon pea is recognized for its high content of bioactive phenolic compounds, including total phenolics, total flavonoids, and strong antioxidant activity. The seed protein of pigeon pea exhibits favorable functional properties such as solubility, water- and oil-absorption capacity, emulsification, and foaming, which enhance its potential for use in various food formulations. (Anjulo et al., 2020; Haji et al., 2024).

C. cajan is widely distributed throughout the tropics as a pulse crop, mainly for grain and also as a cover crop or green manure crop. It is drought-tolerant and has better adaptation to poor soil condition than most tropical legumes. The foliage contains crude protein and fat contents of 20.2% and 1.7%, respectively. These legumes are classified as minor grain legumes because they are underutilized as human food in Nigeria due to the long hours of cooking them before consumption. However, utilization could be expanded because they are sources of dietary protein. They are indigenous and usually cultivated in association with arable crops like yam and cassava. Large biomass of these legume foliage is produced annually (Ajayi, 2011).

C. cajan, among legumes, has an important place in the diet of many people in the world. It is one of the oldest food crops. India alone contributes over 90% of the world's pigeon pea production. It is also a food crop in many other tropical countries and is commercially important in East Africa, the Caribbean, and Latin America. Different parts of this plant are used in traditional medicine in China and Brazil. The antioxidant, antidiabetic, antimicrobial, DNA damage protective and xanthine oxidase inhibitory properties of this plant are generally established. Extracts from this crop are reported to be effective for the treatment of diabetes, dysentery and hepatitis. Leaves have been useful for the treatment of wounds, bedsores, malaria and diet induced hypercholesterolemia, whereas the seeds are sedative and used to treat cough, hepatitis, plasmodial diseases and diabetes. In most of these cases, the effect or molecules need to be identified and characterized. Chemical investigations have been successful in bringing out the major molecules from each part of the plants, especially the leaves and seeds. Leaves are rich in polyphenolic com-pounds such as luteolin and apigenin, flavonoids such as genistein and genistin, anticancerous antiflavone cajanol, stilbenes such as cajaninstilbene acid (CSA) and pinostrobin, antibacterial coumarin cajanuslactone and cajaminose, phenylalanine, and hydroxybenzoic acid with anti-sickle cell disease effects (Mathew et al., 2017).

Considering the escalating global reliance on wastewater irrigation as an alternative water resource, it is imperative to evaluate its implications for soil and water quality, as well as for the safety of crops cultivated under such conditions. Domestic wastewater, derived mainly from household activities, generally contains lower industrial contaminants and provides a richer balance of essential nutrients and organic matter than mixed sewage wastewater, while offering greater soil fertility benefits than treated sewage effluents, which often lose nutrient content during treatment. These characteristics make domestic wastewater a potentially valuable resource for enhancing soil nutrient status, improving microbial activity, and supporting better crop growth and yield with reduced dependence on chemical fertilizers. Accordingly, the present study was undertaken to investigate the environmental and food safety aspects associated with pigeon pea cultivation using domestic sewage wastewater. This research aims to provide scientific insights into the sustainability of wastewater reuse, recycling, and recharge from domestic sewage systems for agricultural purposes, while also elucidating the broader implications for soil health, crop productivity, and public health. The study was conducted in selected urban agricultural fields of Palakkad, Kerala, with a comparative assessment of pigeon pea cultivated under well-water irrigation within the same agroecological zone.

While previous research has often focused on the impact of industrial or mixed wastewater, there is a distinct lack of comprehensive studies assessing the effects of *domestic* sewage wastewater—which has a different nutrient and contaminant profile—on the safety and quality of a multipurpose crop like *C. cajan*. This study aims to fill this knowledge gap by conducting a comparative evaluation of *C. cajan* cultivated using diluted domestic sewage wastewater (semi-urban site) and conventional well water (rural site). We comprehensively assess: (1) water and soil quality parameters, (2) the translocation of essential minerals and heavy metals into various plant tissues, (3) proximate nutritional composition, and (4) the profile of bioactive compounds and associated *in vitro* antioxidant activities. The findings will provide a scientific basis for the safe reuse of domestic wastewater in sustainable legume cultivation, with implications for food security and resource management.

II. MATERIALS AND METHODS

2.1 Plant Sampling Details and Processing:

Cajanus cajan samples were collected from agricultural fields in the Palakkad district of Kerala, representing two distinct irrigation sources:

- **Semi-Urban (SU):** Cultivated using diluted domestic sewage wastewater.
- **Rural (R):** Cultivated using local well water.

From each site, mature plants were collected. Seeds were separated and divided into raw and boiled (100 °C for 15 min) portions. Other plant parts (leaves, stems, pods) were also collected. All samples were washed, oven-dried at 45±5°C, ground, and stored in airtight containers until analysis.

2.2 Water and Soil Sampling and Analysis:

Irrigation water samples were collected in pre-cleaned bottles. Standard physicochemical parameters (pH, EC, TDS, hardness, anions and nutrients) were analyzed as per APHA (2012). Composite soil samples (0-30 cm depth) were collected, processed by quartering, and analyzed for pH, EC, total organic carbon (TOC), available nitrogen (N), phosphorus (P), potassium (K), and heavy metals using standard procedures. were analyzed following the standard procedures outlined in Standard Methods for the Examination of Water and Wastewater (APHA, 2012) Samples were labelled, transported to the laboratory, shade-dried, gently ground with a mortar and pestle, and sieved. A 2 mm sieve was used for general physico-chemical and trace element analysis, while a 0.5 mm sieve was employed for organic carbon estimation. Soil parameters analyzed included pH, EC, total organic carbon (TOC), available nitrogen (Subbiah and Asija, 1956), available phosphorus (Olsen et al., 1954), available potassium (Jackson, 1973), and selected trace and heavy metals, using standard procedures (APHA, 2012).

2.3 Mineral and Heavy Metal Analysis:

Plant, soil, and water samples were digested using tri-acid (HNO_3: H_2SO_4: $HClO_4$, 9:2:1). The concentrations of 17 elements (including Na, Mg, K, Ca, Fe, Zn, Mn, Cu, Ni, Cr, Cd, Pb, As) were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer NexION 300X).

2.4 Proximate Composition Analysis:

The moisture content of raw and processed samples was determined using the Moisture Analyzer MA35 (Sartorius AG, Germany) at 105°C. Crude lipid (Soxhlet extraction), crude fiber and ash contents (gravimetric) were also determined through the methods outlined in the Association of Official Analytical Chemists (AOAC, 1990). The Micro-Kjeldahl method was employed to determine the total nitrogen and a nitrogen protein conversion factor is used for crude protein (N x 6.25) determination. The crude carbohydrate (also called Nitrogen Free Extractives (NFE)) content was estimated by the difference. The proximate composition was expressed as g/100 g DM. The gross energy (KJ) was determined by multiplying the percentage of crude protein, crude lipid and NFE by 16.7, 37.7 and 16.7, respectively (Siddhuraju et al., 1996).

Solvent Extraction for Bioactive Compounds:

Powdered samples were defatted with petroleum ether and subsequently extracted with 70% aqueous acetone (1:7 w/v) at room temperature for 48 h. The crude extracts were filtered, concentrated, and the percentage recovery was calculated.

2.5 Analysis of Bioactive Compounds

- **Total Phenolics and Tannins:** Total phenolic content (TPC) was determined using the Folin–Ciocalteu method (FCM) as described by Siddhuraju and Becker (2003). The FCM measures the reducing capacity of samples and is considered an electron-transfer–based antioxidant assay. Briefly, 100 µl of extract was diluted to 1 ml with distilled water, followed by the addition of 0.5 ml Folin–Ciocalteu reagent (1:1, v/v) and 2.5 ml of 20% sodium carbonate solution. The reaction mixture was vortexed and incubated in the dark for 40 min, and absorbance was recorded at 725 nm against a reagent blank. Analyses were performed in triplicate, and results were expressed as tannic acid equivalents (TAE). Tannin content was estimated after polyvinyl polypyrrolidone (PVPP) treatment according to Siddhuraju and Manian (2007). One hundred milligrams of PVPP was mixed with 1.0 ml distilled water and 1.0 ml phenolic extract, vortexed, and incubated at 40°C for 4 h. After centrifugation at 3000 × g for 10 min, the supernatant containing non-tannin phenolics was analyzed for phenolic content as described above. Tannin content was calculated as: Tannin (%) = Total phenolics (%) − Non-tannin phenolics (%).

- **Total Flavonoids:** Total flavonoid content was determined by the spectrophotometric method of Zhishen et al. (1999) as outlined by Siddhuraju and Becker (2003). An aliquot of 0.1 ml of sample extract or rutin standard (0–100 mg/l) was mixed with 4 ml distilled water in a 10 ml volumetric flask. Subsequently, 0.3 ml of 5% $NaNO_2$ was added, followed after 5 min by 3 ml of 10% $AlCl_3$. At 6 min, 2 ml of 1 M NaOH was added, and the volume was made up to 10 ml with distilled water. Absorbance was measured at 510 nm against a blank. Results were expressed as mg rutin equivalents (RUT) per g extract

In Vitro Antioxidant Activity Assays:

The antioxidant capacity of the extracts was evaluated using five established assays:

1. **Ferric Reducing Antioxidant Power (FRAP):** FRAP activity was measured following the method of Benzie and Strain (1996), as modified by Pulido et al. (2000). The FRAP reagent, freshly prepared and incubated at 37°C, consisted of TPTZ, $FeCl_3·6H_2O$, and acetate buffer (pH 3.6). The assay mixture contained 900 µl FRAP reagent, 90 µl distilled water, and 30 µl extract or methanol (blank). After incubation at 37°C for 30 min, absorbance was measured at 593 nm. $FeSO_4·7H_2O$ (100–2000 µmol/l) was used for calibration. Antioxidant capacity was expressed as mmol Fe(II)/g extract, and EC_1 was defined as the concentration equivalent to 1 mmol/l $FeSO_4·7H_2O$.

2. **Metal Chelating Activity:** Metal chelating activity was determined following the method of Dini et al. (1994). Briefly, 100 µl of extract was mixed with 0.05 ml of 2 mmol/l $FeCl_2$ solution. The reaction was initiated by adding 0.2 ml of 5 mmol/l ferrozine. The mixture was shaken vigorously and allowed to stand at room temperature for 10

min, after which absorbance was measured at 562 nm. Chelating activity was expressed as mg EDTA equivalents per g extract using an EDTA calibration curve (linearity range: 0.5–2.5 µg).

3. **DPPH Radical Scavenging Activity:** Assessed by the ability to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl radical. The radical scavenging activity of sample extracts was measured using DPPH radical by the method of Brand-Williams et al. (1995) with some modifications. An extract of 0.1 mL prepared in methanol was mixed with 3.9 mL of DPPH˙ (6×10-5mol/l methanol) and incubated in the dark for 30 min. Absorbance was read at 515 nm and the results were expressed as mmol trolox equivalents/g extract

4. **ABTS Radical Cation Scavenging Activity:** Measured by the decolorization of the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration (Re et al, 1999), described by (Siddhuraju and Becker, 2003). The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts using the calibration curve of Trolox. The linearity range of the calibration curve was 0.25-1.25 mm/l. The total antioxidant activity of ASC and BHA was also measured by the ABTS•+ method for comparison.

5. **Nitric Oxide (NO) Scavenging Activity:** Nitric oxide scavenging activity was determined using the Griess reaction. Sodium nitroprusside (SNP, 5 mM) in phosphate-buffered saline (pH 7.4) was incubated with different concentrations of sample extracts at 25°C for 150 min. Nitric oxide generated from SNP reacted with oxygen to form nitrite ions, which were quantified by adding Griess reagent (1% sulfanilamide and 0.1% naphthylethylene diamine dihydrochloride in 5% phosphoric acid). Absorbance was measured at 540 nm. Ascorbic acid (ASC) and quercetin (QUE) were used as standards. Nitric oxide scavenging activity was calculated as:

$$\% \text{ Scavenging activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100 \quad (1)$$

Results for FRAP, DPPH, and ABTS were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Metal chelating activity was expressed as mg EDTA equivalent/g extract.

2.6 Statistical Analysis:

All analyses were performed in triplicate. Data are presented as mean ± standard deviation (SD). Significance of differences among means was evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) using SPSS software (Version 21.0).

III. RESULTS AND DISCUSSION

3.1 Physicochemical Quality of Irrigation Water and Soil:

The analysis of irrigation water sources (Table 1) revealed that key parameters for both the semi-urban (diluted sewage) and rural (well water) sources, including pH, electrical conductivity (EC), total dissolved solids (TDS), chloride, and sulphate, were within the permissible ranges prescribed by FAO for irrigation. This indicates a basic suitability for agricultural use. However, water from the semi-urban source showed elevated levels of total alkalinity (254 mg/L) and phosphate (2.4 mg/L), slightly exceeding FAO (1992) thresholds, suggesting nutrient enrichment. Total hardness values (260–540 mg/L) indicated the presence of moderately hard to hard water. A more significant finding was the high concentration of iron (Fe) in both water sources (40.95–41.73 mg/L), far exceeding the FAO limit of 5 mg/L. Crucially, the concentrations of toxic heavy metals of primary concern—Nickel (Ni), Chromium (Cr), Cadmium (Cd), Copper (Cu), Lead (Pb), and Zinc (Zn)—were all below their respective FAO permissible limits, indicating that the diluted sewage was not contaminated with hazardous levels of these industrial pollutants. (FAO, 1992; Bandara et al., 2010). Nickel has been considered to be an essential trace element for human and animal health (Hassan et al., 2012). Higher concentrations of zinc can be toxic to the organism. It plays an important role in protein synthesis and is a metal that shows a fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from the natural sources. Collectively, these findings indicate that while most parameters conform to international irrigation water quality standards, urban water samples are characterized by elevated alkalinity, phosphate, and iron loads, necessitating stringent monitoring and management to mitigate long-term agro-environmental impacts.

TABLE 1
SELECTED PHYSICOCHEMICAL PARAMETERS OF IRRIGATION WATER

Parameter	Unit	Rural (Well Water)	Semi-Urban (Diluted Sewage)	FAO Permissible Limit
pH		7.16	7.02	6.5–8.5
EC	mS/cm	1.09	1.92	0.7–3.0
TDS	mg/L	530	1208	2000
Total Alkalinity	mg/L $CaCO_3$	134	254	250
Phosphate (PO_4^{3-})	mg/L	0.8	2.4	2
Iron (Fe)	**mg/L**	**41.73**	**40.95**	**5**
Nickel (Ni)	mg/L	0.005	0.003	0.2
Chromium (Cr)	mg/L	0.0045	0.005	0.1
Cadmium (Cd)	mg/L	0.0075	0.0075	0.01

Soil analysis (see Supplementary Table S3) indicated near-neutral pH (6.27–6.29), low salinity (EC: 0.08–0.12 dS/m), and moderate fertility. Most importantly, the levels of heavy metals (Ni, Cr, Cu, Pb, Zn, Cd) were found to be orders of magnitude lower than the WHO/FAO permissible limits for agricultural soils. ha and potassium between 490–650 kg/ha, with urban soils recording higher values.

Heavy metal analysis showed that Ni (0.44–0.48 mg/kg), Cr (2.1–5.2 mg/kg), Cu (2.3–4.5 mg/kg), Pb (0.11–0.22 mg/kg), Fe (84–86.5 mg/kg), and Zn (0.82–1.06 mg/kg) were all below the WHO (1996) permissible limits of 80, 100, 30, 100, 200, and 200 mg/kg respectively, except for Fe which was relatively high though still within acceptable agricultural ranges (Iyaka, 2011; Mandal et al., 2011; Wuana and Okieimen, 2011; Khajekar and Deshmukh, 2017; Colombo et al., 2014; Baran et al., 2018). Cd was not detected in any samples. The mineral profiling of C. cajan plant parts revealed Ni (0–0.081 mg/kg), Cr (0–0.02 mg/kg), Cu (0.001–0.13 mg/kg), Zn (0.065–0.74 mg/kg), Mn (0.03–0.30 mg/kg), and Pb (0–0.12 mg/kg) within safe dietary limits (FAO/WHO, 1976; Leshe and Tessema, 2014; Emamverdian et al., 2015). However, Fe content in seeds (96.60–173.19 mg/kg), leaves (143–221.23 mg/kg), stems, and pods consistently exceeded the FAO/WHO (1976) permissible limit of 48 mg/kg, suggesting potential iron accumulation in edible parts of the plant. Overall, while most soil and plant mineral levels remain within international safety limits, elevated iron concentrations pose a significant concern for crop and food safety. This confirms that, under the studied conditions, irrigation with this diluted sewage water had not led to a significant accumulation of toxic metals in the soil profile, mitigating a major long-term risk associated with wastewater reuse.

3.2 Heavy Metal and Essential Mineral Translocation to *C. cajan* Tissues:

The most critical aspect of this study pertains to food safety—the transfer of elements from soil and water into the edible parts of the plant. The analysis of edible seeds (Table 2) yielded reassuring results. Concentrations of potentially toxic heavy metals were all found to be within safe limits for human consumption as per FAO/WHO standards.

- **Toxic Elements:** Cadmium (Cd) was not detected (ND) in any seed sample. The concentrations of Nickel (Ni: 0.01–0.08 mg/kg), Chromium (Cr: 0.01–0.02 mg/kg), and Lead (Pb: 0–0.01 mg/kg) were substantially lower than their respective permissible limits (e.g., 2.3 mg/kg for Cr, 2 mg/kg for Pb).
- **Essential Minerals:** The seeds were confirmed as good sources of essential minerals. Potassium (K) content was high (73.7–954.4 mg/kg), and important micronutrients like Zinc (Zn: 0.07–0.74 mg/kg) and Copper (Cu: 0.01–0.04 mg/kg) were present at safe, nutritionally relevant levels.
- **Elevated Iron Content:** A notable finding was the elevated iron (Fe) content across all seed samples (96.6–173.2 mg/kg), which consistently exceeded the FAO/WHO reference limit of 48 mg/kg (Table 2). This suggests a high natural bioaccumulation propensity for iron in *C. cajan* under the studied conditions, rather than a sign of contamination, a point that warrants further agronomic and physiological investigation.

  The mineral profiling of *C. cajan* revealed distinct patterns of micronutrient and heavy metal accumulation across seeds, leaves, stems, and pods (Table 5). Nickel, although required only in trace quantities for urease activity and nitrogen metabolism (Brown et al., 1987), was present at low levels (0.01–0.081 mg/kg). The maximum concentration was detected in urban boiled seed samples (CCUBS), whereas leaves accumulated only 0.01 mg/kg. These values remain well below the dietary safety range of 3–7 mg/day (Leshe and Tessema, 2014), suggesting no risk of Ni-related toxicity. Chromium, which has not been recognized as an essential element for plants (Shanker et al., 2005), was observed only in seeds (0.01–0.02 mg/kg), with no detectable accumulation in vegetative tissues. All values were far below the FAO/WHO (1976) permissible limit of 2.3 mg/kg, consistent with findings that C. cajan generally exhibits low Cr uptake capacity (Akinyele and Shokunbi, 2015). Cadmium, a non-essential and highly toxic element, was undetectable in all samples, in agreement with previous studies showing limited Cd accumulation in leguminous crops grown under non-contaminated soils (Khan et al., 2017).

Copper concentrations ranged between 0.01–0.04 mg/kg in seeds and 0.001–0.13 mg/kg in vegetative tissues. Although Cu is indispensable for redox regulation and electron transport (Marschner, 2012), its tissue concentrations remained far below the FAO/WHO (1976) dietary limit of 30 mg/kg, minimizing the risk of Cu-induced toxicity. Zinc levels varied between 0.065–0.74 mg/kg in seeds and 0.08–0.30 mg/kg in vegetative tissues, with higher accumulation in CCUBS and rural pod samples. These findings are consistent with earlier reports indicating that pH and soil composition strongly influence Zn bioavailability (Emamverdian et al., 2015). In plants, Cu plays key roles in photosynthesis, respiration, cell wall metabolism and hormone perception. Multiple Cu transporters regulate the transport of Cu. (Majhi and Sikdar, 2023). Manganese concentrations were comparatively low (0.04–0.07 mg/kg in seeds; 0.03–0.30 mg/kg in vegetative tissues), yet the highest accumulation occurred in leaves, reflecting Mn's role in chloroplast function and photosystem II activity (Millaleo et al., 2010). Importantly, all Mn values remained within the tolerable daily intake of 11 mg/day (Leshe and Tessema, 2014). Iron exhibited markedly elevated concentrations (96.60–173.19 mg/kg in seeds; 143.0–221.23 mg/kg in vegetative tissues), significantly surpassing the FAO/WHO (1976) permissible limit of 48 mg/kg. This trend suggests a strong capacity of *C. cajan* for Fe bioaccumulation, particularly in rural leaves (CCRL, 221.23 mg/kg). While iron enrichment may be beneficial in addressing anemia in iron-deficient populations, excessive levels can disrupt the uptake of other essential micronutrients and pose risks of iron overload (Abbaspour et al., 2014).

Lead was detected only in trace amounts in urban raw seed (0.013 mg/kg), rural pod (0.01 mg/kg), and urban pod (0.12 mg/kg) samples. These concentrations were well below the FAO/WHO (1976) safety threshold of 2 mg/kg, suggesting minimal Pb contamination, despite its well-known persistence and cumulative toxicity (Jarup, 2003). Overall, *C. cajan* demonstrated nutritionally relevant concentrations of essential trace elements such as Zn, Mn, Cu, and Ni, with negligible contamination by Cd, Cr, and Pb. However, the consistently high Fe concentrations warrant further investigation into soil–plant interactions, potential sources of Fe enrichment, and implications for long-term dietary exposure. These results align with previous studies highlighting legumes as both a valuable nutritional source and a potential sink for soil-derived heavy metals (Alloway, 2013). Wastewater may have beneficial or harmful effects on crop production, depending upon its composition and the sensitivity of the plant species (Uzma et al., 2016).

TABLE 2
MINERAL AND HEAVY METAL PROFILE OF *C. CAJAN* SEEDS (mg/kg dry weight)

Element	Rural Raw Seed	Rural Boiled Seed	Semi-Urban Raw Seed	Semi-Urban Boiled Seed	FAO/WHO Reference Limit*
Potassium (K)	954.4	650.64	731.4	73.72	–
Iron (Fe)	158.41	173.19	96.6	150.17	**48**
Zinc (Zn)	0.103	0.065	0.56	0.74	60
Copper (Cu)	0.04	0.02	0.03	0.01	30
Manganese (Mn)	0.075	0.07	0.05	0.04	–
Nickel (Ni)	0.054	0.041	0.01	0.081	–
Chromium (Cr)	0	0	0.01	0.02	2.3
Lead (Pb)	0	0	0.013	0	2
Cadmium (Cd)	0	0	0	0	0.2

3.3 Proximate Nutritional Composition:

Proximate composition analysis of *C. cajan* was conducted using standard methods to evaluate its nutritional potential, including moisture, ash, crude lipid, crude fibre, crude protein, nitrogen-free extract (crude carbohydrate), and energy value per 100 g (Table 3). The results revealed marked variations among plant parts and between urban and rural samples. Moisture content, an important factor for storage stability (Nielsen, 2010), was generally higher in rural samples, indicating greater susceptibility to post-harvest deterioration. (Oke, 2014; Blazos and Belski, 2016; Chukwu et al., 2013). Seeds were characterized by high carbohydrate and protein levels, confirming their value as an energy-dense legume food (Sebastia et al., 2001).

Boiling reduced lipid, carbohydrate, and energy values but slightly improved protein content, consistent with earlier reports on processing effects in legumes (Pattee et al., 2009; Yellavila et al., 2015). Overall, the findings highlight C. cajan seeds as major sources of energy and protein, while vegetative parts, particularly leaves, contribute significantly to fibre, minerals, and lipids. Proximate analysis (Table 3) confirmed that *C. cajan* seeds from both irrigation sources are a nutritionally dense food. These values are comparable to earlier studies on legume crops (Kamboj and Nanda, 2017).Carbohydrates (as Nitrogen-Free Extract, NFE) were the predominant component (67.0–69.4 g/100g), typical for legumes. The crude protein content was substantial and comparable between sources, ranging from 15.5 to 17.6 g/100g. Crude fiber (3.2–4.8 g/100g) and crude lipid (6.1–7.2 g/100g) contents were also notable. Boiling, a common processing step, caused minor but significant reductions in some components like lipids and certain bioactive compounds due to leaching, but it did not drastically alter the core nutritional profile. Importantly, there was no consistent negative effect attributable to the sewage irrigation source; for instance, semi-urban boiled seeds exhibited slightly higher protein content than their rural counterpart.

TABLE 3
PROXIMATE COMPOSITION OF *C. CAJAN* SEEDS (g/100g dry matter)

Component	Rural Raw Seed	Rural Boiled Seed	Semi-Urban Raw Seed	Semi-Urban Boiled Seed
Moisture (%)	8.16	7.67	6.53	7.16
Crude Ash	4.62	4.19	4.52	4.14
Crude Protein	15.5	17.04	16.25	17.57
Crude Lipid	6.76	6.14	7.22	6.62
Crude Fiber	3.77	4.78	3.15	4.7
Carbohydrate (NFE)	69.35	67.85	68.86	66.98
Gross Energy (kJ/100g)	**1671.86**	**1649.11**	**1693.52**	**1661.4**

3.4 Bioactive Compounds and *In Vitro* Antioxidant Activity:

- **Extract Yield and Phenolic Content:** The yield of the 70% acetone extract varied among samples. Rural raw seeds yielded the highest extract (18.3%). Total phenolic content (TPC) was significant and generally higher in rural seeds (96.9 mg TAE/g extract) than in semi-urban seeds (62.8 mg TAE/g). Boiling reduced TPC due to thermal degradation and leaching. Analysis of non-seed tissues revealed them to be richer reservoirs of bioactive compounds than seeds. Leaves and pods, in particular, exhibited significantly higher levels of total phenolics (up to 326.2 mg TAE/g extract in rural pods) and antioxidant capacity (see Supplementary Table S4), underscoring their potential value as sources of nutraceuticals or for use in animal feed.

  Extraction of bioactive compounds is a crucial initial step for utilizing phytochemicals from *C. cajan* in nutraceutical, food, pharmaceutical, and cosmetic applications. Although nutritionally valuable, its use is constrained by anti-nutritional factors such as trypsin inhibitors, hemagglutinins, and saponins (Solomon et al., 2017). Solvent extraction efficiency depends on solvent polarity, extraction conditions, and sample characteristics (Dai and Mumper, 2010). Extract yields ranged from 10–18.3% in seeds, with higher yields in raw than processed samples, indicating losses due to processing, while vegetative parts—especially leaves—showed markedly higher yields (17.6–34.2%). Total phenolic content was highest in rural raw seeds and substantially reduced by boiling, whereas non-seed tissues, particularly pods, contained significantly higher phenolics, highlighting their antioxidant potential (Khattab et al., 2009; Xu and Chang, 2008; Balasundram et al., 2006). Tannin and flavonoid contents followed similar trends, with processing reducing concentrations due to thermal degradation and leaching (Khandelwal et al., 2010; Rani and Fernando, 2016). Overall, rural samples consistently exhibited higher levels of phenolics, tannins, and flavonoids, likely influenced by environmental factors that enhance secondary metabolite synthesis (Ali et al., 2013), emphasizing the importance of optimized processing and the valorization of non-seed plant parts.

- **Antioxidant Capacity:** All *C. cajan* extracts exhibited considerable *in vitro* antioxidant power across all five complementary assays (Table 4), confirming a robust and multifaceted antioxidant potential. A strong positive correlation was observed between the total phenolic content of the extracts and their activity across all five antioxidant assays (FRAP, DPPH, ABTS, Metal Chelating, NO Scavenging), confirming phenolics as the primary contributors to the observed antioxidant potential. Among seeds, rural raw samples consistently showed the highest activity (e.g., FRAP: 12,924.6 mmol Fe(II)/g; DPPH: 20,423.4 mmol TE/g). While the synthetic antioxidants BHA and ascorbic acid demonstrated higher absolute activity, the natural activity displayed by *C. cajan* extracts is promising for health and nutritional applications. The high antioxidant potential, maintained despite differing irrigation sources, underscores the nutraceutical value and resilience of pigeon pea.

The antioxidant activity of *C. cajan* extracts, evaluated using FRAP, metal chelating, DPPH, ABTS, and nitric oxide radical scavenging assays, demonstrated marked variation between raw and processed seeds as well as among different plant parts. The FRAP assay, widely applied for assessing reducing power in botanicals (Nithiyanantham et al., 2012), showed higher activity in raw samples than processed ones, indicating processing-induced losses of electron-donating antioxidants, consistent with earlier reports on legumes (Sathya and Siddhuraju, 2013).

Metal chelating activity, which reflects secondary antioxidant potential by stabilizing metal ions and preventing redox cycling (Joy et al., 2017; Siddhuraju et al., 2014), was also reduced after processing but not completely abolished. DPPH and ABTS radical scavenging assays revealed that boiling adversely affected antioxidant capacity due to leaching and thermal degradation of soluble antioxidants and polyphenols (Nithiyanantham et al., 2012; Siddhuraju et al., 2014). Nitric oxide scavenging activity further confirmed higher efficacy in raw samples, with reductions attributed to losses of polyphenols, vitamins, and minerals during thermal treatment (Boora et al., 2014; Dhanya et al., 2019). Although all plant extracts exhibited lower antioxidant activity compared to standards such as ascorbic acid and BHA, the observed trends strongly correlated with phenolic content, reinforcing that phenolic compounds are the principal contributors to antioxidant capacity in C. cajan (Irik and Bikmaz, 2024).

TABLE 4
ANTIOXIDANT ACTIVITY OF *C. CAJAN* SEED EXTRACTS

Sample	FRAP (mmol Fe(II)/g)	Metal Chelating (mg EDTA/g)	DPPH (TEAC mmol/g)	ABTS (TEAC mmol/g)	NO Scavenging (%)
Rural Raw Seed	12924.58 ± 35.9	2.78 ± 0.02	20423.36 ± 52.6	3903.74 ± 62.8	68.73
Rural Boiled Seed	8289.79 ± 75.5	2.67 ± 0.01	20350.36 ± 43.8	3665.42 ± 64.7	55.97
Semi-Urban Raw Seed	3869.14 ± 50.1	2.73 ± 0.01	15951.34 ± 22.3	18992.85 ± 186.5	59.35
Semi-Urban Boiled Seed	3764.45 ± 51.5	1.47 ± 0.01	15323.60 ± 36.7	18101.75 ± 189.5	NA
Standards					
Ascorbic Acid	730676.32 ± 91.2	–	493310.06 ± 1125.9	597116.67 ± 104.8	–
BHA	350278.70 ± 73.6	10.49 ± 0.06	386368.04 ± 9622.1	654356.06 ± 617.1	–

IV. CONCLUSION

This comprehensive study provides robust evidence that the use of diluted domestic sewage wastewater for irrigating *C. cajan*, under the specific conditions of this preliminary field assessment, did not result in the dangerous accumulation of toxic heavy metals (Cd, Pb, Cr, Ni, Cu) in the edible seeds. All measured values remained well within international food safety standards.

The irrigation water, while nutrient-rich and high in iron, was not contaminated with problematic levels of industrial pollutants. The proximate nutritional quality of the seeds—their protein, carbohydrate, and energy content—was maintained and was comparable to seeds grown with well water.

Furthermore, the plants retained significant levels of bioactive phenolic compounds and associated high *in vitro* antioxidant activity, which was strongly correlated. The elevated iron content in seeds warrants further study but appears to be a varietal or physiological trait rather than a contamination issue. The non-seed biomass (leaves, pods) showed particularly high phenolic content, indicating valuable by-products.

Therefore, with necessary precautions—primarily the ongoing monitoring of water quality (particularly for salts and Fe) and soil health—the controlled use of diluted domestic sewage wastewater can be considered a viable and sustainable irrigation alternative for pigeon pea cultivation. This practice supports critical goals of water conservation, nutrient recycling, and urban food security without compromising the safety or nutraceutical value of this important legume crop. Further long-term studies under varied field conditions are recommended to validate these promising findings.

AUTHOR CONTRIBUTIONS

- **Dhanya Viswanathan**: methodology, investigation, data curation, formal analysis, writing–original draft.
- **Velukutty Amrutha:** investigation, data curation, conceptualization, writing – review and editing.
- **Perumal Siddhuraju:** methodology, conceptualization, data curation, supervision, project administration, writing – review and editing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (P. Siddhuraju*, Email: psiddhuraju@buc.edu.in) upon reasonable request.

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