

GROWTH PERFORMANCE, MINERAL COMPOSITION AND HEAVY METAL ACCUMULATION IN JAMBO GRASS (*Sorghum bicolor* × *S. sudanense*) IRRIGATED WITH INDUSTRIAL EFFLUENT

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Supporting Information



ABSTRACT: The use of industrial wastewater for forage production is increasing in peri-urban livestock systems, yet its implications for fodder safety remain uncertain. This study evaluated the effects of graded industrial effluent irrigation on growth characteristics, nutrient composition, and heavy metal accumulation in Jambo grass (*Sorghum bicolor* × *Sorghum sudanense*). A pot experiment was conducted using five irrigation treatments comprising fresh water (control) and 25%, 50%, 75%, and 100% industrial effluent. Morphological traits, biomass yield, proximate composition, and concentrations of Pb, Cd, Cr, Ni, Cu, Zn, and Fe were determined in soil and plant tissues. Moderate effluent application (25–50%) improved plant height, leaf production, and total biomass yield compared with the control. However, progressive increases in effluent concentration resulted in elevated accumulation of Pb and Cd, particularly in roots and leaves. At higher effluent levels (≥75%), Pb and Cd concentrations in edible plant parts exceeded recommended limits for livestock feed, despite proximate composition remaining largely unaffected. Crude protein and fiber fractions showed no significant variation among treatments. The results indicate that diluted industrial effluent may support Jambo grass productivity, but unrestricted use poses potential risks to livestock health due to heavy metal contamination. Careful dilution and routine monitoring are therefore essential if wastewater-irrigated fodder is to be used in animal feeding systems.

Keywords: Forage quality, Jambo grass, Heavy metals, Livestock feed safety, Wastewater irrigation.

INTRODUCTION

Industrial effluent discharge has become a persistent challenge in many livestock-producing regions, particularly where agricultural land is located near industrial zones (Qadir et al., 2010; Aftab et al., 2023; Islam et al., 2023). In such areas, wastewater is frequently used for irrigation due to limited freshwater availability and its perceived nutrient value (Pedrero et al., 2010; Ungureanu et al., 2020). While industrial effluents may supply essential minerals that support plant growth, they often contain potentially toxic elements that can accumulate in soil and fodder crops (Nagajyoti et al., 2010; Alloway, 2013; Penzy et al., 2023; Islam et al., 2023).

Forage crops grown under contaminated irrigation are of particular concern because they serve as the primary feed source for ruminant animals (Khan et al., 2008; Othman et al., 2021). Heavy metals such as lead and cadmium are not biologically degradable and may persist in plant tissues, ultimately entering animal products (McLaughlin et al., 1999; Islam et al., 2023). Chronic exposure, even at low concentrations, can impair animal performance and pose risks to food safety (NRC, 2005; Atta et al., 2022). Despite these concerns, limited information is available on the response of commonly cultivated fodder grasses to industrial effluent irrigation under controlled conditions.

Jambo grass (*Sorghum bicolor* × *Sorghum sudanense*) is widely used in tropical livestock systems due to its high biomass yield, adaptability, and suitability for cut-and-carry feeding (FAO, 2011). Its rapid growth and extensive root system suggest a degree of tolerance to suboptimal soil and water conditions (Skerman and Riveros, 1990). However, information on its mineral uptake behavior and heavy metal accumulation under effluent irrigation remains scarce, although previous findings on morphological responses of fodder crops under industrial effluent stress have been documented (Islam et al., 2023).

Therefore, the present study was undertaken to evaluate the effects of industrial effluent irrigation on growth performance, nutrient composition, and heavy metal accumulation in Jambo grass, with specific emphasis on its suitability as a livestock feed resource.

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MATERIALS AND METHODS

Experimental location and climatic conditions

The experiment was conducted at the Livestock and Poultry Farm of Gazipur Agricultural University (GAU), Gazipur, Bangladesh. The experimental period (February to June, 2024) represented the transition from late winter to early monsoon, marked by increasing temperature, relative humidity, and occasional rainfall, which are conducive to enhanced growth of irrigated fodder crops (BBS, 2023). All experimental procedures were conducted in accordance with the methodology described by Islam et al. (2023), ensuring consistency with established protocols.

Soil collection and pot preparation

Surface soil (0-15 cm) was collected from the university research field, air-dried, crushed, and sieved to remove debris. Plastic pots (36 × 36 × 32.5 cm) were filled with 18 kg of prepared soil. During pot preparation, well-decomposed cow dung was incorporated at 400 g per pot to improve soil organic matter and nutrient availability. Basal fertilization was applied using urea, triple superphosphate, and muriate of potash at 10, 15, and 12 g per pot, respectively, two weeks before sowing. The fertilizer rates were selected based on recommended nutrient requirements for sorghum-sudangrass hybrids and adjusted proportionally to pot soil weight to ensure balanced nutrient supply under controlled conditions (FAO, 2011; Havlin et al., 2014; FRG, 2018).

Planting material and experimental design

Jambo grass (*Sorghum bicolor* × *S. sudanense*) seeds were obtained from the Department of Animal Nutrition, Bangladesh Agricultural University. Three seeds were sown per pot in a triangular arrangement. The experiment followed a completely randomized design with five irrigation treatments and eight replications.

Irrigation treatments

Industrial effluent was collected from a nearby garment factory discharge point within the university area. During the irrigation period, effluent samples were consistently collected from the same location of a local drainage channel situated near the Livestock and Poultry Farm, GAU, and were applied directly for irrigation. The collected effluent was thoroughly mixed before application to ensure homogeneity. The effluent was used as irrigation water at five different concentrations (0%, 25%, 50%, 75%, and 100%) by mixing with fresh water according to the treatment design. Water samples (industrial effluent and fresh water) were collected weekly, preserved in clean plastic containers, and stored at 4 °C for subsequent laboratory analysis. Five irrigation treatments were established using different proportions of fresh water and industrial effluent: T₁: 100% fresh water; T₂: 75% fresh water + 25% effluent; T₃: 50% fresh water + 50% effluent; T₄: 25% fresh water + 75% effluent; T₅: 100% effluent. All pots received equal volumes of irrigation water at 3-day intervals, depending on moisture conditions.

Crop management and harvesting

Routine intercultural operations, including weeding and fencing, were conducted throughout the experimental period. No pesticides were applied. Jambo grass was harvested 75 days after sowing. Fresh biomass from each pot was weighed immediately after harvest.

Measurement of growth parameters

Plant height, number of tillers, number of leaves, leaf length, leaf yield, and stem yield were recorded following harvest. The leaf-to-stem ratio was calculated from dry matter yield.

Sample preparation and laboratory analysis

Irrigation water analysis

Irrigation water samples were collected and stored in plastic bottles that had been pre-washed with 10 mol/L HCl, rinsed thoroughly with deionized water, and air-dried. The collected samples were filtered through a 0.45 µm membrane filter using a 100 mL syringe and subsequently stored in clean plastic bottles. To prevent precipitation and preserve heavy metals and trace elements, the samples were acidified by adding 10 mol/L HCl to each 50 mL of water sample. The effluent samples were analyzed following standard analytical methods as described by Thomas (1982), Hossain et al. (2015).

Soil and plant sample analysis

Soil and plant samples were collected from each pot. The soil samples were air-dried, cleaned, homogenized, and ground using a grinding machine, then passed through a 20-mesh sieve. Similarly, the plant samples were oven-dried at 65 °C for approximately 48 hours until a constant weight was achieved, after which they were ground and sieved through a 20-mesh sieve. For digestion, 0.5 g of both soil and plant samples were treated with a di-acid mixture of HNO₃-HClO₄ (2.5:1) following the method of Norvell et al. (2000) using a block digester (Model: VELP). After adding the acid mixture,

samples were left overnight and subsequently heated at 120 °C for about 4 hours until the evolution of white fumes. The digested samples were then filtered through Whatman No. 1 filter paper, and the volume was adjusted to 50 mL with deionized water. The prepared extracts were used for the determination of heavy metals (Pb, Cd, Cr, Ni, Cu, Zn, and Fe). The concentrations of these metals were analyzed using an atomic absorption spectrophotometer equipped with a Buck Scientific 210 VGP continuous flow hydride generation system (HG-AAS). The proximate composition of plant samples was analyzed following the method of AOAC (2019) and expressed on a dry matter basis.

Statistical analysis

Data were analyzed using analysis of variance appropriate for a completely randomized design. Treatment means were compared using Duncan's multiple range test, and differences were considered significant at $P < 0.05$.

RESULTS

Heavy metal and mineral composition of fresh water and industrial effluent before irrigation

Industrial effluent exhibited significantly higher ($P < 0.01$) concentrations of Pb, Cd, Cr, Ni, Cu, Fe, Mg, Na, K, and Ca compared to fresh water used for irrigation (Table 1). In contrast, no significant differences ($P > 0.05$) were observed between fresh water and industrial effluent with respect to Zn and Mn concentrations.

Soil heavy metal and mineral status after harvest

Post-harvest soil analysis revealed significant effects of effluent irrigation on Pb ($P < 0.01$), Cd ($P < 0.01$), Ni ($P < 0.01$), Fe ($P < 0.05$), Mg ($P < 0.01$), K ($P < 0.05$), and Ca ($P < 0.01$) concentrations (Table 2). Lead and cadmium levels increased with effluent application, particularly at higher concentrations. Nickel showed maximum accumulation under the 50% effluent treatment; moreover, iron and magnesium showed higher accumulation under the 100% effluent treatment (Table 2). Chromium, copper, zinc, manganese, and sodium did not differ significantly ($P > 0.05$) among treatments. However, after harvest, there was minimal accumulation of potassium and calcium in the soil.

Growth and biomass yield of Jambo grass

Plant height differed significantly ($P < 0.01$) among treatments, with the tallest plants observed under 25% effluent irrigation. Total biomass yield was highest ($P < 0.01$) at 50% effluent and lowest under 100% effluent irrigation. The number of tillers showed no significant ($P > 0.05$) variation, while leaf number and leaf length were significantly higher under moderate effluent treatments (Table 3). Leaf yield increased ($P < 0.01$) with effluent dilution up to 50%, whereas stem yield declined progressively at higher effluent (100%) concentrations. The leaf-to-stem ratio increased ($P < 0.01$) with increasing effluent proportion.

Heavy metal accumulation in plant tissues

The concentrations of heavy metals and macro-minerals in different parts of Jambo grass were influenced to varying degrees by industrial effluent irrigation (Table 4). Lead (Pb) concentration in whole grass remained unaffected ($P > 0.05$), although significant differences were observed in plant parts, with higher accumulation in leaves under T_4 ($P < 0.01$), stems under T_5 ($P < 0.05$), and roots under T_3 ($P < 0.01$). Cadmium (Cd) concentration in whole grass did not vary significantly ($P > 0.05$), whereas leaf Cd content differed markedly ($P < 0.01$), showing the highest value in T_1 and the lowest in T_5 ; root Cd accumulation was also significant ($P < 0.01$), with greater values in T_1 and T_4 . Chromium (Cr) remained statistically unchanged across all treatments and plant parts ($P > 0.05$). Nickel (Ni) concentration varied significantly in whole grass and all plant components ($P < 0.01$), with maximum accumulation in whole grass and roots under T_3 , in leaves under T_5 , and in stems under T_2 and T_3 . Copper (Cu) concentration showed no significant variation in whole grass and stems ($P > 0.05$), but differed in leaves ($P < 0.05$) and roots ($P < 0.01$), with relatively higher values under moderate effluent treatments. Zinc (Zn) concentration decreased significantly in whole grass with increasing effluent levels ($P < 0.01$), with the highest value in T_1 , while significant differences were also observed in leaves ($P < 0.01$), stems ($P < 0.05$), and roots ($P < 0.01$), with root Zn peaking under T_3 . Iron (Fe) concentration showed a slight but significant reduction in whole grass at higher effluent levels ($P < 0.05$), whereas significant variation was observed in leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with maximum accumulation generally under intermediate to higher effluent treatments. Among macro-minerals, magnesium (Mg) concentration in whole grass did not differ significantly ($P > 0.05$), although significant variation was observed in leaves ($P < 0.05$), stems ($P < 0.01$), and roots ($P < 0.01$), with stem Mg increasing under effluent application and root Mg declining with increasing effluent levels. Manganese (Mn) concentration decreased significantly in whole grass ($P < 0.05$), while significant differences were observed in leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with maximum accumulation generally recorded under T_3 . Sodium (Na) concentration was significantly affected in whole grass and all plant parts ($P < 0.01$), with higher values in control and higher effluent treatments, depending on plant component. Potassium (K) concentration showed highly significant variation ($P < 0.01$) in all plant parts, with whole grass and root K increasing under higher effluent levels, while leaf and stem responses varied among treatments. Calcium (Ca) concentration differed significantly in whole grass ($P < 0.05$), leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with increased accumulation generally observed under moderate effluent treatments. Overall, industrial effluent irrigation significantly influenced the distribution of both heavy metals and essential minerals in Jambo grass, with distinct partitioning patterns among leaves, stems, and roots.

Table 1 - Heavy metal and mineral composition of irrigation water used in the experiment (ppm)

Component (ppm)	Irrigation water	Fresh water	Waste water	P-value*
Pb		0.031±0.03 ^b	3.508±0.68 ^a	0.000
Cd		0.023±0.01 ^b	1.639±0.29 ^a	0.000
Cr		0.002±0.00 ^b	3.082±0.72 ^a	0.000
Ni		0.038±0.02 ^b	5.193±1.05 ^a	0.000
Cu		0.759±0.28 ^b	3.596±0.93 ^a	0.000
Zn		0.228±0.1	0.300±0.07	0.136
Fe		0.564±0.21 ^b	1.396±0.35 ^a	0.000
Mg		2.958±0.97 ^b	5.574±1.01 ^a	0.000
Mn		5.672±0.95	5.887±1.03	0.692
Na		7.025±0.68 ^b	8.566±0.51 ^a	0.000
K		1.509±0.42 ^b	4.017±0.26 ^a	0.000
Ca		2.653±0.21 ^b	4.645±0.83 ^a	0.000

*P-values indicate differences between fresh water and industrial effluent.

Table 2 - Heavy metal and mineral composition of soil after harvest as influenced by industrial effluent irrigation (ppm)

Component (ppm)	Treatment*	T ₁	T ₂	T ₃	T ₄	T ₅	P-value
Pb		3.27±0.10 ^b	3.39±0.11 ^b	3.99±0.12 ^a	3.86±0.16 ^a	3.26±0.14 ^b	0.0001
Cd		0.35±0.10 ^b	0.46±0.11 ^{ab}	0.34±0.10 ^b	0.64±0.10 ^a	0.66±0.08 ^a	0.005
Cr		0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.04±0.01	0.646
Ni		3.19±0.17 ^c	3.17±0.25 ^c	4.20±0.15 ^a	3.88±0.20 ^{ab}	3.47±0.14 ^{bc}	0.0001
Cu		0.36±0.03	0.47±0.04	0.43±0.06	0.43±0.09	0.43±0.09	0.467
Zn		2.19±0.19	2.10±0.12	2.05±0.12	1.96±0.07	2.20±0.09	0.168
Fe		28.25±1.04 ^{ab}	27.24±1.03 ^b	28.75±1.11 ^{ab}	29.20±0.77 ^{ab}	30.44±0.39 ^a	0.017
Mg		377.48±2.29 ^c	338.68±2.96 ^d	374.47±3.86 ^c	417.19±4.30 ^b	471.82±2.41 ^a	0.000
Mn		0.28±0.03	0.28±0.06	0.35±0.03	0.30±0.08	0.31±0.05	0.464
Na		203.62±4.56	201.93±3.08	196.31±1.60	200.39±1.89	200.38±3.06	0.119
K		125.65±1.63 ^a	118.58±4.16 ^b	118.48±3.13 ^b	118.48±3.09 ^b	123.79±2.97 ^{ab}	0.041
Ca		265.06±3.33 ^a	225.42±1.69 ^c	204.46±4.29 ^e	214.46±4.05 ^d	242.61±3.77 ^b	0.000

*T₁: 100% fresh water; T₂: 75% fresh water + 25% effluent; T₃: 50% fresh water + 50% effluent; T₄: 25% fresh water + 75% effluent; T₅: 100% industrial effluent. Means within a row bearing different superscripts differ significantly (p < 0.05)

Table 3 - Effect of industrial effluent irrigation on morphological traits and biomass yield of Jambo grass at 75 days

Parameters	Treatment*	T ₁	T ₂	T ₃	T ₄	T ₅	P-value
Plant height (cm)		102.58±3.35 ^b	114.33±2.42 ^a	106.92±4.36 ^b	106.00±4.20 ^b	103.67±4.37 ^b	0.000
No of Tillers/ Plant		4.33±0.56	3.89±0.81	3.89±0.50	3.83±0.41	3.61±0.65	0.355
Number of leaves/ Plant		17.44±1.33 ^b	21.11±2.56 ^a	18.17±2.34 ^{ab}	18.67±2.07 ^{ab}	17.00±2.13 ^b	0.023
Longest Leaf length (cm)		64.00±2.52 ^{ab}	66.94±2.54 ^a	66.06±2.40 ^a	65.72±2.65 ^{ab}	61.89±2.10 ^b	0.012
Leaf wide (cm)		6.08±0.40	6.98±0.39	6.78±0.86	6.96±0.66	6.22±0.86	0.073
Biomass Yield /plant (gm)		151.44±7.76 ^b	165.44±6.83 ^b	196.00±12.31 ^a	155.56±8.57 ^b	98.56±8.86 ^c	0.000
Leaf Yield/ Plant (gm)		40.24±2.62 ^d	70.67±4.74 ^b	92.33±5.99 ^a	52.44±4.94 ^c	56.00±4.61 ^c	0.00
Stem Yield/ Plant (gm)		294.50±4.66 ^a	238.33±3.27 ^c	254.67±4.03 ^b	215.67±4.03 ^d	131.67±4.80 ^e	0.00
Leaves to stem ratio		0.53±0.03 ^d	0.78±0.07 ^c	0.91±0.04 ^b	0.73±0.09 ^c	1.28±0.14 ^a	0.000

*T₁: 100% fresh water; T₂: 75% fresh water + 25% effluent; T₃: 50% fresh water + 50% effluent; T₄: 25% fresh water + 75% effluent; T₅: 100% industrial effluent. Values are mean ± SD. Different superscripts within a row indicate significant differences (p < 0.05).

Proximate composition of Jambo grass

Moisture content showed a non-significant ($P = 0.06$) increasing trend with higher effluent levels. Crude protein, crude fiber, crude fat, ash, nitrogen-free extract, neutral detergent fiber, and acid detergent fiber did not differ significantly among treatments ($P > 0.00$). All proximate values remained within typical ranges for tropical forage grasses (Table 5).

Table 4 - Heavy metal and mineral concentration in different parts of Jambo grass under varying irrigation treatments (ppm).

Component (ppm)	Different parts	*Treatment					P-value
		T ₁	T ₂	T ₃	T ₄	T ₅	
Pb	Whole grass	4.20±0.53	3.74±0.24	4.00±0.51	3.82±0.24	3.89±0.12	0.61
	Leaf	3.54±0.08 ^c	3.87±0.08 ^b	3.87±0.11 ^b	4.08±0.08 ^a	3.75±0.05 ^b	0.00
	Stem	4.26±0.28 ^{ab}	4.09±0.12 ^{ab}	4.18±0.09 ^{ab}	3.83±0.23 ^b	4.49±0.12 ^a	0.017
	Root	3.59±0.02 ^d	4.15±0.12 ^c	4.66±0.04 ^a	4.19±0.05 ^c	4.49±0.06 ^b	0.000
Cd	Whole grass	0.48±0.20	0.48±0.27	0.36±0.24	0.36±0.11	0.33±0.06	0.78
	Leaf	0.75±0.04 ^a	0.37±0.05 ^{bc}	0.37±0.06 ^{bc}	0.47±0.06 ^b	0.29±0.03 ^c	0.00
	Stem	0.72±0.08	0.66±0.05	0.67±0.13	0.78±0.22	0.84±0.08	0.417
	Root	1.04±0.02 ^a	0.73±0.17 ^b	0.73±0.14 ^b	1.10±0.06 ^a	0.61±0.04 ^b	0.001
Cr	Whole grass	6.56±0.60	0.02±0.01	0.02±0.01	0.02±0.00	0.02±0.01	0.85
	Leaf	0.02±0.00	0.03±0.01	0.03±0.01	0.02±0.00	0.02±0.01	0.56
	Stem	0.04±0.00	0.05±0.01	0.05±0.02	0.03±0.03	0.03±0.02	0.487
	Root	0.02±0.01	0.03±0.01	0.04±0.00	0.04±0.02	0.02±0.01	0.304
Ni	Whole grass	4.40±0.62 ^d	4.89±0.45 ^{cd}	6.57±0.29 ^a	5.33±0.41 ^{bc}	5.71±0.25 ^b	0.00
	Leaf	6.24±0.22 ^b	5.92±0.31 ^{bc}	4.64±0.19 ^d	5.56±0.17 ^c	6.93±0.12 ^a	0.00
	Stem	5.99±0.20 ^b	6.94±0.10 ^a	6.89±0.20 ^a	6.33±0.15 ^b	5.10±0.21 ^c	0.000
	Root	4.90±0.22 ^c	4.33±0.08 ^d	7.71±0.10 ^a	6.29±0.19 ^b	4.99±0.13 ^c	0.000
Cu	Whole grass	0.80±0.23	0.88±0.05	0.73±0.06	0.89±0.19	0.88±0.07	0.60
	Leaf	0.86±0.08 ^a	0.75±0.05 ^{ab}	0.83±0.03 ^a	0.84±0.07 ^a	0.64±0.05 ^b	0.01
	Stem	0.90±0.03	0.94±0.05	0.86±0.07	0.92±0.10	0.92±0.14	0.803
	Root	0.87±0.11 ^{ab}	0.71±0.02 ^c	0.92±0.02 ^a	0.81±0.02 ^b	0.89±0.02 ^{ab}	0.005
Zn	Whole grass	24.29±0.5 ^a	21.39±0.7 ^b	22.23±0.36 ^b	22.32±0.5 ^b	19.96±0.4 ^c	0.00
	Leaf	24.40±0.37 ^a	23.08±0.1 ^b	20.31±0.36 ^d	22.18±0.1 ^c	23.66±0.14 ^b	0.00
	Stem	23.43±0.16 ^{ab}	21.76±0.8 ^b	22.81±1.44 ^{ab}	24.98±0.92 ^a	23.79±0.37 ^{ab}	0.012
	Root	19.11±0.84 ^c	22.65±2.28 ^b	26.04±0.09 ^a	21.85±0.21 ^b	23.64±1.07 ^{ab}	0.000
Fe	Whole grass	320.00±1.68 ^a	319.97±2.13 ^a	316.77±1.94 ^a	316.52±4.71 ^a	310.84±1.95 ^b	0.01
	Leaf	308.25±3.21 ^c	329.17±0.78 ^a	316.93±1.88 ^b	310.05±1.14 ^c	303.73±1.13 ^d	0.00
	Stem	300.53±2.60 ^c	318.70±4.23 ^b	307.76±4.38 ^c	331.42±1.59 ^a	331.03±3.16 ^a	0.000
	Root	319.21±3.07 ^{ab}	314.82±2.79 ^{bc}	311.29±3.74 ^c	325.51±3.20 ^a	309.98±2.11 ^c	0.001
Mg	Whole grass	172.00±1.17	174.99±1.97	176.03±1.78	176.11±2.74	176.84±2.83	0.13
	Leaf	177.01±2.99 ^{ab}	173.64±0.43 ^{bc}	174.86±0.78 ^{abc}	171.61±0.36 ^c	177.81±0.74 ^a	0.00
	Stem	176.88±2.86 ^c	201.11±1.04 ^a	191.28±2.34 ^b	189.18±3.06 ^b	194.22±2.15 ^b	0.000
	Root	203.36±0.42 ^a	195.63±3.47 ^b	183.02±1.17 ^c	175.66±2.90 ^d	180.12±2.56 ^{cd}	0.000
Mn	Whole grass	9.90±0.62 ^a	8.87±0.19 ^b	9.03±0.30 ^b	8.68±0.28 ^b	8.55±0.12 ^b	0.01
	Leaf	9.42±0.15 ^a	8.97±0.12 ^b	9.44±0.09 ^a	8.50±0.15 ^c	9.47±0.13 ^a	0.00
	Stem	5.24±0.27 ^b	4.39±0.56 ^b	6.22±0.29 ^a	4.49±0.54 ^b	4.89±0.37 ^b	0.002
	Root	4.86±0.13 ^b	4.90±0.13 ^b	6.26±0.06 ^a	4.98±0.19 ^b	4.17±0.16 ^c	0.000
Na	Whole grass	468.24±3.92 ^a	442.03±3.35 ^c	451.64±2.49 ^b	432.95±2.16 ^d	441.37±3.27 ^c	0.00
	Leaf	477.13±2.79 ^a	439.23±1.15 ^c	434.89±1.94 ^c	435.24±1.96 ^c	465.41±1.60 ^b	0.00
	Stem	472.93±3.13 ^b	434.80±3.42 ^d	449.12±4.30 ^c	497.48±2.98 ^a	468.18±3.94 ^b	0.000
	Root	472.75±3.94 ^a	430.73±1.82 ^c	422.51±2.47 ^d	468.60±3.47 ^a	452.37±2.22 ^b	0.000
K	Whole grass	844.85±3.78 ^{bc}	841.61±3.64 ^c	849.23±2.12 ^b	827.32±2.38 ^d	930.78±2.31 ^a	0.00
	Leaf	952.54±1.88 ^a	784.25±1.10 ^d	817.94±3.26 ^c	910.68±1.26 ^b	816.96±2.35 ^c	0.00
	Stem	810.37±3.04 ^e	984.01±3.70 ^a	937.02±4.29 ^b	878.32±4.41 ^c	858.79±3.03 ^d	0.000
	Root	932.17±3.18 ^d	985.97±3.89 ^c	998.14±4.53 ^b	796.39±4.09 ^e	1048.02±3.65 ^a	0.000
Ca	Whole grass	144.49±4.14 ^b	144.90±3.61 ^b	146.35±2.10 ^b	153.41±3.06 ^a	145.15±3.09 ^b	0.04
	Leaf	141.84±1.53 ^c	153.77±0.81 ^b	162.16±0.85 ^a	142.96±1.18 ^c	142.91±1.32 ^c	0.00
	Stem	149.06±2.19 ^b	157.14±1.91 ^{ab}	150.56±4.18 ^b	149.25±3.63 ^b	161.33±3.80 ^a	0.003
	Root	152.92±2.47 ^d	164.02±3.05 ^b	172.37±3.12 ^a	147.86±0.43 ^d	158.87±0.53 ^c	0.000

*T₁: 100% fresh water; T₂: 75% fresh water + 25% effluent; T₃: 50% fresh water + 50% effluent; T₄: 25% fresh water + 75% effluent; T₅: 100% industrial effluent. Whole grass values represent composite samples. Means with different superscripts differ significantly ($p < 0.05$).

Table 5 - Proximate and fiber composition of Jambo grass under different irrigation treatments (% DM).

Parameters (%)	T ₁	T ₂	T ₃	T ₄	T ₅	P-value
Moisture	18.71±1.18	18.68±0.32	19.55±0.79	21.14±1.11	19.57±1.09	0.06
Crude protein	7.48±0.32	6.22±0.24	6.56±0.60	6.70±0.57	7.01±0.80	0.13
Crude fiber	30.86±0.75	32.04±1.51	31.36±1.51	30.43±1.62	31.70±1.57	0.67
Crude fat	1.01±0.06	1.09±0.03	0.93±0.16	0.96±0.06	0.96±0.13	0.43
Ash	8.95±0.86	8.40±0.90	8.53±0.47	8.67±0.70	8.67±0.36	0.89
NFE	32.98±1.36	33.58±2.01	33.06±0.34	32.09±0.97	32.08±0.79	0.53
NDF	63.28±2.20	62.42±1.53	65.71±0.99	64.98±2.64	63.80±2.05	0.32
ADF	44.09±1.08	45.77±2.15	44.80±2.15	43.47±2.31	45.28±2.25	0.67

*T₁: 100% fresh water; T₂: 75% fresh water + 25% effluent; T₃: 50% fresh water + 50% effluent; T₄: 25% fresh water + 75% effluent; T₅: 100% industrial effluent. DM, dry matter; NFE, nitrogen-free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.

DISCUSSION

Moderate industrial effluent irrigation enhanced the growth and biomass production of Jambo grass, while higher concentrations induced physiological stress. Thus, industrial effluent irrigation exerted a dual effect on the growth and nutrient dynamics of Jambo grass. The observed enhancement in plant height, leaf development, and biomass under moderate effluent (25-50%) application can be attributed to the increased availability of essential nutrients such as potassium, calcium, and magnesium, which commonly enrich soil fertility and contribute to improved plant metabolism and growth (Marschner, 2012; Yerli et al., 2025).

The decline in biomass and stem yield at higher effluent levels indicates a shift from nutrient benefit to toxicity, likely associated with excessive accumulation of heavy metals such as Pb, Cd, and Ni, which are known to disrupt root function, physiological processes, enzyme activity, and nutrient uptake processes (Shanker et al., 2005; Ali et al., 2013; Nowwar et al., 2023; Islam et al., 2023). Nickel and zinc showed maximum accumulation under moderate effluent levels (50%), suggesting that metal availability and plant uptake efficiency are enhanced at intermediate contamination levels. Yadav et al. (2002), who noted that moderate metal concentrations could stimulate uptake mechanisms before reaching toxicity thresholds, reported similar observations. In contrast, the decline in Cd and Zn concentrations at higher effluent levels (100%) may be attributed to physiological stress and reduced root permeability, which limits metal absorption under toxic conditions (Kabata-Pendias, 2000; Nagajyoti et al., 2010). The predominance of heavy metal accumulation in roots observed in the present study reflects an inherent protective tolerance mechanism whereby plants restrict the translocation of metals to aerial tissues, a phenomenon widely documented in forage species exposed to metal stress (Sharma et al., 2006; Yusuf et al., 2011). However, this physiological barrier may become progressively less effective with prolonged exposure or elevated effluent concentrations, leading to increased metal movement into shoots at higher treatment levels, as reported in recent findings (Kanwal et al., 2024).

From a feed safety perspective, the elevated concentrations of Pb and Cd detected in leaves under higher effluent treatments (100%) are of particular concern, as wastewater-irrigated crops have been reported to accumulate toxic metals beyond safe limits, thereby posing risks to livestock and human health through food chain transfer (Sharma et al., 2015; Singh et al., 2024). The recommended maximum levels in complete livestock diets are generally 8 mg kg⁻¹ for Pb and 0.4 mg kg⁻¹ for Cd (0.2 mg kg⁻¹ for horses), thresholds established to minimize toxicity and residue transfer into edible animal products (CFIA, 2024). Moreover, from a livestock nutrition perspective, exceeding these thresholds may impair feed intake, growth, reproduction, renal and hepatic function in animals, while chronic consumption can lead to toxicity, reduced productivity, and the transfer of contaminants into animal-derived products such as milk, meat, and offal (Food and Agriculture Organization of the United Nations and World Health Organization, 2001; Faye and Sinyavskiy, 2008), thereby elevating human dietary exposure.

In contrast, essential micronutrients such as Zn, Cu, Fe, and Mn remained within acceptable limits, indicating that wastewater irrigation may enhance certain aspects of mineral nutrition without compromising overall forage quality, as also reported in similar studies on wastewater-irrigated fodder crops (Yadav et al., 2009; Bhatnagar et al., 2020); however, long-term application may still lead to bioaccumulation and associated health risks, particularly for elements such as Zn when present in excess (Akhtar et al., 2025). According to FAO (2001) guidelines, elevated levels of heavy metals in forage crops pose risks to animal productivity and food safety. The relatively stable proximate composition across treatments further supports the notion that basic nutritional attributes are less sensitive to effluent-induced changes than mineral composition.

Overall, these findings confirm a threshold response, where moderate effluent use can be beneficial for forage production, but higher concentrations result in heavy metal accumulation and potential toxicity. Nevertheless, the imbalance between beneficial nutrients and toxic metals under higher effluent levels underscores a critical threshold beyond which the risks outweigh the agronomic advantages. Therefore, the findings highlight the importance of controlled dilution and continuous monitoring of industrial effluent when used for forage production to ensure both productivity and feed safety within sustainable livestock systems.

CONCLUSION

Industrial effluent irrigation influenced both growth performance and mineral accumulation in Jambo grass. Moderate effluent dilution improved biomass production without markedly altering proximate composition. However, elevated concentrations of lead and cadmium in plant tissues at higher effluent levels raise concerns regarding fodder safety. The findings indicate that while diluted industrial effluent may be used cautiously for forage production, unrestricted application is not advisable. Regular monitoring of heavy metal concentrations is essential before wastewater-irrigated fodder is incorporated into livestock feeding systems.

DECLARATIONS

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Ethical approval

This study did not involve the use of live animals or human participants.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authors' contributions

Shilpi Islam conceived, designed, and implemented the experiment. Shilpi Islam and Susmita Sarker Bristi participated in data collection and laboratory analyses. Md. Rashidul and Abu Sadek Md. Selim performed the statistical analysis and interpretation of the results. Shilpi Islam prepared the initial draft of the manuscript. All authors contributed to manuscript revision, read, and approved the final version.

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Consent for publication

All authors have reviewed and approved the final manuscript and consent to its publication.

Competing Interests

The authors declare that they have no competing interests.

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