Sugar Metabolism as an Indicator of Plant Energy Status in Hydrocarbon-Stressed Soils Amended with Cassava Mill Effluent

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Abstract

This study investigated sugar metabolism as a physiological indicator of plant energy status in *Vigna unguiculata* grown in hydrocarbon-contaminated soils amended with cassava mill effluent (CME). Soils contaminated with 10% spent lubricating oil (SLO) were treated with CME at varying frequencies (once, monthly, weekly, daily) before cowpea cultivation. Glucose concentrations were quantified in leaves, shoots, and roots at 28 days using the Nelson-Somogyi method. Hydrocarbon contamination severely disrupted sugar metabolism, but CME amendment induced tissue-specific recovery patterns. In leaves, glucose levels increased dramatically in amended polluted soils, with polluted soil treated daily (PTD) showing 10528.91 µg/gfw (63.7-fold increase) and polluted soil treated once (PTN)14426.13 µg/gfw (87.3-fold increase) compared to general control (GC - unpolluted, unamended) (165.33 µg/gfw). Shoot glucose exhibited contrasting patterns, with polluted soil treated monthly (PTM) showing 117.39 µg/gfw (62.58% reduction) while polluted control (PC - polluted, unamended) maintained 252.75 µg/gfw. Root glucose showed variable responses: PTN increased by 404.64% (482.69 µg/gfw) while PTM showed only 76.33% increase (291.44 µg/gfw). The differential tissue responses reveal complex allocation strategies under combined hydrocarbon and amendment stresses. Leaf sugar accumulation suggests enhanced photosynthetic production or altered phloem loading, while shoot reductions may indicate preferential carbon allocation to defense mechanisms over growth. Root variability reflects complex belowground carbon dynamics involving exudation, storage, and microbial interactions. Glucose patterns correlated with plant growth parameters, antioxidant status, and hydrocarbon degradation rates, supporting its utility as an integrative indicator of plant metabolic health in remediation contexts. These findings demonstrate that sugar metabolism provides sensitive insights into plant energy status and adaptation strategies in amended contaminated soils, with potential applications for monitoring phytoremediation efficacy and optimizing amendment protocols.

Keywords: Sugar metabolism, glucose dynamics, energy status, hydrocarbon stress, *Vigna unguiculata*, carbon allocation, phytoremediation monitoring

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Introduction

Plant metabolism under environmental stress represents a complex integration of physiological responses aimed at maintaining cellular homeostasis, energy balance, and growth potential (Taiz *et al.,* 2015). Among metabolic parameters, sugar dynamics serve as particularly sensitive indicators of plant energy status, reflecting the balance between photosynthetic carbon fixation, respiratory energy expenditure, and allocation to defense and growth processes (Rolland *et al.,* 2006). Under optimal conditions, plants maintain tightly regulated sugar homeostasis, but environmental stressors including soil contamination by petroleum hydrocarbons can profoundly disrupt this balance, with cascading effects on plant health, growth, and survival (Chen *et al.,* 2020).

Hydrocarbon contamination affects plant sugar metabolism through multiple mechanisms: direct inhibition of photosynthetic electron transport and Calvin cycle enzymes, disruption of chloroplast integrity, and induction of metabolic pathways that divert carbon resources toward defense compounds rather than growth (Shah *et al.,* 2017). The resulting energy imbalance can compromise essential physiological processes, limit growth and reproduction, and ultimately determine plant survival in contaminated environments (Singh *et al.,* 2018). Understanding how plants adjust their sugar metabolism under hydrocarbon stress and how these adjustments can be supported through soil amendments is therefore critical for developing effective phytoremediation strategies and for assessing plant health during remediation processes.

Cassava mill effluent (CME) amendment has shown promise for supporting plant establishment in hydrocarbon-contaminated soils through improvement of soil physicochemical properties and reduction of contaminant toxicity (Olukanni *et al.,* 2019; Jude *et al.,* 2022). However, the specific effects of CME on plant metabolic processes—particularly sugar metabolism and energy status—under pollution stress remain poorly understood. As some organic amendment rich in carbohydrates and nutrients, CME could potentially influence plant sugar dynamics through several pathways: providing additional carbon substrates, improving nutrient availability for metabolic enzymes, reducing hydrocarbon toxicity, and stimulating microbial activity that affects rhizosphere carbon cycling.

Sugar metabolism responses are often tissue-specific, reflecting different functional priorities and stress exposures in leaves, shoots, and roots (Koch, 2004). Leaves, as primary photosynthetic organs, must balance carbon fixation with defense compound synthesis. Shoots, responsible for transport and structural support, allocate

resources between growth and defense. Roots, interfacing directly with contaminated soil, must manage both stress responses and symbiotic relationships while maintaining essential uptake functions. Understanding these tissue-specific responses provides a more complete picture of plant adaptation strategies in amended contaminated soils. Glucose, as a central metabolite in plant carbon metabolism, serves as a key indicator of sugar status and energy availability (Smeckens, 2000). Its concentration reflects the integration of photosynthetic production, respiratory consumption, transport efficiency, and storage mobilization. Changes in glucose levels can indicate shifts in metabolic priorities, energy limitations, or compensatory adjustments to environmental challenges. In the context of hydrocarbon contamination and organic amendment, glucose dynamics may reveal how plants allocate limited carbon resources among competing demands: growth versus defense, aboveground versus belowground investment, immediate survival versus long-term adaptation.

This study addresses these knowledge gaps through detailed investigation of glucose metabolism in *Vigna unguiculata* grown in CME-amended hydrocarbon-contaminated soils. By examining tissue-specific glucose concentrations in relation to amendment frequency, plant growth parameters, antioxidant status, and soil conditions, this research provides insights into plant energy management strategies under combined contamination and amendment stresses. The findings contribute to developing sugar metabolism as a physiological indicator for monitoring plant health in remediation contexts while advancing fundamental understanding of plant metabolic adaptations to contaminated environments.

Materials and Methods

Soil Collection and Characterization

The soil used in this experiment was collected from a fallow agricultural field at the University at 0-20 cm depth. The soil was hand-picked manually to remove debris and stones, and homogenized prior to analysis and experimental setup.

The pre-experimental physicochemical properties of the soil were determined using standard procedures (Table 1). Soil pH was measured in a 1:2.5 soil-water suspension using a digital pH meter (Jenway 3510). Electrical conductivity (EC) was determined using a conductivity meter. Particle size distribution was analyzed by the hydrometer method (Bouyoucos, 1962). Organic carbon content was determined by Walkley-Black dichromate oxidation method (Walkley and Black, 1934). Total nitrogen was measured by the Kjeldahl digestion method (Bremner and Mulvaney, 1982). Available phosphorus was extracted using Bray-1 solution and determined colorimetrically. Exchangeable cations (Ca, Mg, K, Na) were extracted with 1N ammonium acetate (pH 7.0) and quantified using atomic absorption spectrophotometry (AAS). Cation exchange capacity (CEC) was determined by summation of exchangeable bases and acidity (Sumner and Miller, 1996). Total hydrocarbon content (THC) was extracted with n-hexane and quantified spectrophotometrically.

Cassava Mill Effluent (CME) Characterization

Fresh cassava mill effluent was collected from a cassava processing facility in Benin City. The effluent was characterized for physicochemical properties essential for understanding its amendment effects (Table 2). The CME composition was analyzed following standard methods (APHA, 2017). Cyanide content was estimated by alkaline picrate method (Onwuka, 1996) distillation and titration method. Nutrient concentrations (N, P, K, Ca, Mg) were determined using standard procedures.

Table 1: Pre-experimental Physicochemical Properties of Soil

Parameter	Value
Sand (%)	72.4 ± 2.3
Silt (%)	15.6 ± 1.2
Clay (%)	12.0 ± 0.9
Textural class	Sandy loam
pH $_{(H_2O)}$	6.42 ± 0.15
Electrical conductivity (dS/m)	0.18 ± 0.02
Organic carbon (%)	1.24 ± 0.11
Total nitrogen (%)	0.11 ± 0.01
Available phosphorus (mg/kg)	8.45 ± 0.67
Exchangeable K (cmol/kg)	0.28 ± 0.03
Exchangeable Ca (cmol/kg)	2.64 ± 0.21
Exchangeable Mg (cmol/kg)	1.12 ± 0.09
Exchangeable Na (cmol/kg)	0.14 ± 0.02
CEC (cmol/kg)	5.86 ± 0.42
Total hydrocarbon content (mg/kg)	24.6 ± 2.1

Values are mean ± standard error of three replicates

Table 2: Physicochemical Properties of Cassava Mill Effluent Used for Amendment

Parameter	Value
pH	4.38 ± 0.12
Electrical conductivity (dS/m)	1.86 ± 0.14
Total solids (g/L)	28.4 ± 2.1
Total nitrogen (mg/L)	245 ± 18
Total phosphorus (mg/L)	38.6 ± 3.2
Potassium (mg/L)	412 ± 24
Calcium (mg/L)	186 ± 15
Magnesium (mg/L)	94.5 ± 7.8
Cyanide (mg/L)	12.4 ± 1.1
Total carbohydrates (g/L)	15.8 ± 1.2

Values are mean ± standard error of three replicates

Experimental Design and Plant Cultivation

The experiment utilized soils contaminated with 10% (w/w) spent lubricating oil (SLO) and amended with CME at 2.5% (v/w) concentration with frequencies of once only (PTN), monthly (PTM), weekly (PTW), and daily (PTD). Unamended polluted (PC) and unpolluted controls (GC, TN, TM, TW, TD) were maintained for comparison. After six months of soil amendment, cowpea (*Vigna unguiculata* (L.) Walp., local variety) was cultivated and monitored under natural environmental conditions for 28 days with regular watering to maintain approximately 70% field capacity.

Tissue Sampling and Preparation

At harvest (28 days), plants were carefully uprooted and separated into leaves, shoots and roots. Tissues were immediately washed with distilled water, blotted dry,

weighed and were macerated using a mortar and pestle with appropriate solvent.

Glucose Extraction and Quantification

Glucose was extracted using the method of Nelson (1944) and Somogyi (1945) with modifications. Fresh macerated tissue (0.5 g) was extracted with 10 mL of 80% ethanol at 80°C for 30 minutes with occasional stirring. The extract was centrifuged at 5000 × g for 15 minutes, and the supernatant was collected. The residue was re-extracted twice with 5 mL of 80% ethanol and the supernatant was pulled together.

Glucose concentration was determined using the Nelson-Somogyi copper reduction method. Briefly, 1 mL of tissue extract was mixed with 1 mL Somogyi reagent and heated in a boiling water bath for 10 minutes. After cooling, 1 mL Nelson's reagent was added and shaken vigorously. The tubes were allowed to stand for 10 minutes and the mixture was diluted to 10 mL with distilled water. Absorbance was measured at 600 nm using a spectrophotometer (Techmel & Techmel 20D). Glucose concentration was calculated from a standard curve prepared with analytical grade D-glucose (0-500 µg/mL). Results were expressed as µg glucose per gram fresh weight (µg/gfw).

Correlative Analyses

Glucose concentrations were correlated with previously determined parameters from the same experimental plants: plant growth measurements (height, biomass, leaf area), photosynthetic pigments (chlorophyll a, b, carotenoids), antioxidant enzyme activities (SOD, CAT), oxidative stress markers (MDA, proline, ascorbic acid), and soil properties (pH, EC, CEC, nutrient levels, hydrocarbon content). These parameters were measured concurrently from the same experimental units using standard methods.

Statistical Analysis

The experimental design followed a 2 × 5 factorial structure with two pollution levels (unpolluted and polluted with 10% SLO) and five amendment treatments (unamended control, once, monthly, weekly, daily CME application). Data were analyzed using two-way analysis of variance (ANOVA) to evaluate main effects of pollution, amendment frequency, and their interaction. This factorial approach allows assessment of: 1. Main effects of pollution on glucose metabolism 2. Main effects of amendment frequency on glucose metabolism 3. Pollution × amendment interaction effects, which are biologically central to understanding how CME amendments perform specifically under contaminated conditions Where significant differences were detected, means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test ($p < 0.05$). For specific comparisons of interest, treatment effects were assessed relative to general control (GC) for unpolluted treatments and relative to polluted control (PC) for polluted treatments. Correlation coefficients (Pearson's r) were calculated between glucose levels and other measured parameters. Principal component analysis (PCA) was performed to identify patterns in multivariate data. All statistical analyses were performed using GraphPad Prism version 9.4 and SPSS version 25, with results presented as mean ± standard error of three replicates.

Treatment Codes

The following treatment codes were used throughout the study:

GC = General control (unpolluted, unamended), TN = Treated once (unpolluted), TM = Treated monthly (unpolluted), TW = Treated weekly (unpolluted), TD = Treated daily (unpolluted), PC = Polluted control (polluted, unamended), PTN = Polluted + treated once, PTM = Polluted + treated monthly, PTW = Polluted + treated weekly, PTD = Polluted + treated daily.

Results

Two-Way ANOVA Results

Two-way ANOVA revealed significant main effects and interactions for all tissues (Table 3). Pollution had highly significant effects on glucose content in all tissues ($p < 0.001$). Amendment frequency significantly affected glucose in leaves and roots ($p < 0.001$) but showed a weaker effect on shoots ($p < 0.05$). Critically, the pollution × amendment interaction was significant for all tissues ($p < 0.001$ for leaves and roots; $p < 0.01$ for shoots), confirming that the effect of CME amendment depends fundamentally on whether soils are polluted or unpolluted. This interaction justifies the factorial analytical approach and demonstrates that amendment strategies optimized in clean soils cannot be directly extrapolated to contaminated conditions.

Tissue-Specific Glucose Dynamics

Table 1 reveals striking tissue-specific carbon allocation in cowpea under hydrocarbon stress. Leaves showed extreme glucose accumulation in amended polluted soils 87.3-fold (PTN) and 63.7-fold (PTD) above GC (165.33 µg/gfw). This aligns with Chen *et al.* (2020) and Gururani *et al.* (2021), who reported hydrocarbon-induced sugar accumulation from impaired phloem loading and disrupted sucrose transporters. CME may enhance photosynthesis while hydrocarbon inhibits sugar export (Saddique *et al.,* 2022). Conversely, shoot glucose declined consistently, with PTM showing 62.58% reduction. This reflects growth-defense tradeoffs (Huot *et al.,* 2014), mediated by trehalose-6-phosphate signaling (Figueroa *et al.,* 2023). Negative correlation with SOD ($r = -0.73$) confirms carbon diversion to antioxidant synthesis (Méndez-Espinoza *et al.,* 2021). Root glucose varied: PTN increased 404.64%, PTM only 76.43%. Pulsed nutrients stimulate temporary root carbon allocation (Canarini *et al.,* 2019). Correlations with soil pH ($r = 0.53$) and CEC ($r = 0.49$) suggest improved soil conditions support root metabolism (Wang et al., 2022).

Metabolic Implications of Leaf Glucose Accumulation

Leaf glucose (8,567-14,426 µg/gfw) indicates potential metabolic dysfunction. Concentrations >10,000 µg/gfw signal feedback inhibition of photosynthesis and disrupted sugar sensing (Rolland *et al.,* 2006). Li *et al.* (2021) reported similar accumulation with Calvin cycle

Table 3: Glucose Content in *Vigna unguiculata* Tissues at 28 Days (µg/g fresh weight)

Treatment	Leaves (µg/gfw)	% Change vs GC	Shoots (µg/gfw)	% Change vs GC	Roots (µg/gfw)	% Change vs GC
GC	165.33 ± 12.45	0	313.67 ± 18.22	0	95.67 ± 7.33	0
TN	423.56 ± 25.67	156.18	287.44 ± 16.89	-8.36	178.44 ± 12.45	86.56
TM	567.89 ± 34.12	243.49	256.78 ± 15.67	-18.14	291.44 ± 18.33	204.64
TW	689.45 ± 42.33	317.02	234.56 ± 14.89	-25.22	345.67 ± 22.45	261.33
TD	745.67 ± 48.90	351.02	198.67 ± 12.34	-36.66	412.33 ± 28.90	331.08
PC	4233.56 ± 256.78	2460.84	252.75 ± 16.78	-19.43	156.78 ± 11.23	63.89
PTN	14426.13 ± 867.45	8625.67	189.56 ± 12.45	-39.57	482.69 ± 34.56	404.64
PTM	8567.89 ± 512.34	5082.56	117.39 ± 8.90	-62.58	168.78 ± 12.34	76.43
PTW	9234.56 ± 567.89	5485.67	156.78 ± 10.23	-50.02	234.56 ± 17.89	145.22
PTD	10528.91 ± 634.56	6268.45	178.89 ± 11.45	-42.98	201.67 ± 15.67	110.84

Table 4: Percentage Changes in Glucose Content Relative to Respective Controls

Treatment	Leaves vs PC	Shoots vs PC	Roots vs PC	Overall Pattern
PTN	240.67%	-25.00%	207.89%	Leaf/Root accumulation
PTM	102.44%	-53.55%	7.66%	Leaf-specific accumulation
PTW	118.11%	-37.96%	49.67%	Moderate accumulation
PTD	148.67%	-29.22%	28.67%	Balanced response

Table 5: Two-Way ANOVA Results for Glucose Content in Cowpea Tissues

Source of Variation	Leaves		Shoots		Roots	
	F-value	p-value	F-value	p-value	F-value	p-value
Pollution (P)	3245.67	<0.001	98.45	<0.001	156.78	<0.001
Amendment (A)	156.89	<0.001	4.23	<0.05	67.34	<0.001
P × A Interaction	89.56	<0.001	6.78	<0.01	42.56	<0.001

Table 6: Correlation Coefficients (Pearson's r) Between Glucose Levels and Other Parameters

Parameter	Leaves Glucose	Shoots Glucose	Roots Glucose	Significance
Plant Height	0.78**	-0.65*	0.42	$p < 0.01$
Leaf Area	0.82**	-0.71**	0.38	$p < 0.01$
Total Biomass	0.75**	-0.68*	0.45*	$p < 0.05$
Chlorophyll a	0.69*	-0.62*	0.51*	$p < 0.05$
Chlorophyll b	0.72**	-0.59*	0.48*	$p < 0.05$
SOD Activity	0.85**	-0.73**	0.56*	$p < 0.01$
CAT Activity	0.63*	-0.55*	0.42	$p < 0.05$
MDA Content	-0.77**	0.66*	-0.39	$p < 0.01$
Proline	0.81**	-0.69*	0.47*	$p < 0.01$
Ascorbic Acid	0.88**	-0.76**	0.58*	$p < 0.01$
Soil THC	-0.92**	0.81**	-0.61*	$p < 0.01$
Soil pH	0.74**	-0.67*	0.53*	$p < 0.01$
Soil CEC	0.79**	-0.70**	0.49*	$p < 0.01$

**$p < 0.01$, *$p < 0.05$*

Table 7: PCA Loadings for First Three Principal Components

Parameter	PC1 (48.7%)	PC2 (23.4%)	PC3 (12.8%)
Leaves Glucose	0.92	0.18	-0.12
Shoots Glucose	-0.87	0.24	0.31
Roots Glucose	0.64	-0.52	0.43
Plant Height	0.78	0.41	-0.23
Leaf Area	0.81	0.38	-0.19
SOD Activity	0.85	0.22	-0.31
Ascorbic Acid	0.89	0.15	-0.25
Soil THC	-0.94	0.11	0.18
Soil pH	0.76	-0.43	0.32
Treatment Frequency	0.68	0.56	-0.28

downregulation. Positive correlation with ascorbic acid (r = 0.88) suggests glucose fuels antioxidant synthesis (Foyer, 2024). However, excessive hexose triggers hexokinase-mediated photosynthetic repression (Tognetti *et al.,* 2023). Single applications caused highest accumulation; frequent applications lower, supporting that pulsed nutrients disrupt homeostasis more than sustained inputs (Smith *et al.,* 2022).

Shoot Glucose Reduction

PTM showed severe shoot glucose reduction (-62.58%) despite good growth. Metabolomic profiling reveals reallocation invisible to growth metrics (Gargallo-Garriga *et al.,* 2018). Negative correlations with SOD (r = -0.73) and AsA (r = -0.76) confirm strategic defense allocation, not energy limitation. Defense responses demand substantial carbon (Züst & Agrawal, 2017). Hydrocarbon-stressed Populus allocate 40% of fixed carbon to defense, reducing shoot sugars (Wang *et al.,* 2023). PTM's minimal root increase but severe shoot reduction suggests optimal defense without excessive rhizodeposition.

Root Glucose Dynamics

PTN's strong root increase (404.64%) may reflect enhanced belowground allocation or impaired utilization. Hydrocarbon initially stimulates root sugar accumulation, then depletion (Vives-Peris *et al.,* 2020). Weak THC correlation (r = -0.61) indicates indirect effects via soil properties are more critical than direct toxicity (Ossai *et al.,* 2022). Rhizodeposition responds to both plant stress and microbial activity (Pausch & Kuzyakov, 2018).

Glucose as Remediation Indicator

Table 4 shows leaf glucose correlates strongly with soil THC (r = -0.92), directly reflecting hydrocarbon stress. Al-Hawas *et al.* (2023) found leaf sugars correlated better with sediment hydrocarbons than growth parameters. Positive correlations with SOD (r = 0.85) and AsA (r = 0.88) link glucose accumulation to defense capacity. Nadarajah (2024) proposed soluble sugars as integrative phytoremediation biomarkers. Negative MDA correlation (r = -0.77) confirms effective defense reduces oxidative damage. PCA (Table 5) shows glucose parameters load strongly on PC1 (48.7%) with antioxidants and soil THC, confirming sugar metabolism's centrality to stress response (Sarker & Oba, 2021).

Frequency-Dependent Responses and Interaction Effects

The significant pollution × amendment interaction detected by two-way ANOVA ($p < 0.001$) confirms that the effect of CME on leaf glucose depends fundamentally on whether soils are contaminated. In unpolluted soils, CME produced modest, balanced increases (2.6-4.5 fold) typical of nutritional stimulation (Iqbal *et al.,* 2020). In polluted soils, identical amendments caused extreme accumulation (51.8-87.3 fold), indicating that hydrocarbon contamination fundamentally alters how plants perceive and respond to organic amendments, possibly through modification of hexokinase-dependent glucose sensing pathways (Rasheed *et al.,* 2022).

Single applications (PTN) caused extreme disruption: 240.67% higher leaf glucose, 207.89% higher root glucose than PC. Pulsed high-rate applications cause greater metabolic disturbance than frequent low-rate inputs due to temporary nutrient excess and osmotic stress (Diacono & Montemurro, 2019). Weekly and monthly applications produced balanced responses. PTM showed efficient allocation: pronounced shoot reduction (-53.55%) but modest leaf and root accumulation. Moderate-frequency amendments optimize nutrient provision while avoiding negative effects (Agegnehu *et al.,* 2021). PTW and PTD intermediate patterns support weekly/monthly applications as optimal.

Integration with Plant Performance

Leaf glucose positively correlates with growth (height: r = 0.78, leaf area: r = 0.82) yet shoot glucose negatively correlates a paradox resolved by source-sink imbalance indices (White *et al.,* 2024). Positive chlorophyll correlations (r = 0.69-0.72) indicate glucose accumulation does not reflect photosynthetic inhibition. Hydrocarbon-tolerant plants maintain photosynthesis through compensatory mechanisms supported by CME nutrients (Yadav *et al.,* 2023).

Comparative Analysis: Polluted vs Unpolluted Systems

In polluted soils, the same amendments caused fundamentally different responses: extreme leaf accumulation (5,000-8,600% above GC) and the non-linear frequency response where PTN > PTD > PTW > PTM. This context-dependency, confirmed by significant pollution × amendment interactions in two-way ANOVA, demonstrates that hydrocarbon contamination fundamentally alters plant metabolic regulation. Rasheed et al. (2022) reviewed evidence that hydrocarbons modify sugar sensing and signaling pathways, potentially causing inappropriate metabolic responses to otherwise beneficial inputs. This finding has practical implications: amendment strategies optimized in clean soils cannot be directly transferred to contaminated systems without context-specific testing.

Conclusion

This study demonstrates that sugar metabolism in *Vigna unguiculata* undergoes dramatic, tissue-specific alterations under hydrocarbon stress and CME amendment, providing sensitive insights into plant energy status and adaptation strategies. Hydrocarbon contamination induces extreme leaf glucose accumulation (8,567-14,426 µg/gfw), correlating strongly with soil THC (r = -0.92), establishing leaf glucose as a sensitive biomarker of contaminant exposure. Shoot glucose shows consistent reductions (up to 62.58%) with negative correlations to antioxidant enzymes (SOD: r = -0.73), confirming strategic carbon reallocation from growth to defense. Root glucose exhibits treatment-specific variability (7-405% changes), reflecting complex belowground interactions. Two-way ANOVA revealed significant pollution × amendment interactions for all tissues ($p < 0.001$), confirming that amendment effects depend fundamentally on pollution context. This finding has critical implications for phytoremediation practice: strategies developed in clean soils cannot be directly extrapolated to contaminated conditions. Amendment frequency critically determines metabolic outcomes. Weekly and monthly applications generate the most balanced tissue-specific responses—pronounced shoot

reduction without pathological leaf accumulation—and are therefore optimal for phytoremediation support.

Recommendation

Implementation of tissue-specific glucose monitoring as routine phytoremediation assessment. Establish threshold values: leaf glucose <5,000 µg/gfw = adaptive; 5,000-10,000 = severe stress requiring intervention; >10,000 = metabolic dysfunction warranting protocol modification. Optimization of CME application using glucose feedback, with weekly or monthly applications at 2.5% (v/w) producing optimal responses. Integration of glucose assessment with physiological and soil parameters for comprehensive monitoring. Development of field-deployable glucose testing using adapted handheld meters for rapid, low-cost analysis.

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