UJMR, Vol. 10 No. 2, December, 2025, pp. 11 - 22 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.25102.002

Received: 23/08/2025 **Accepted:** 02/11/2025

Morpho-physiological Responses of Sunflower (*Helianthus annus* L) Exposed to Lead and Cyanide Stress

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Abstract

Listeria monocytogenes is a critical food-borne pathogen responsible for listeriosis, a severe infection that has significant repercussions on global public health. Considering the dearth of relevant researches on the prevalence and antibiogram of this pathogen in Northeastern Nigeria, this research was carried out to highlight the resilience of the pathogen against the backdrop of the rise of antimicrobial resistance. The bacteria were isolated from 258 food samples collected from diverse locations and various food types within Maiduguri, Borno State. Isolation was conducted using modified Public Health England protocols, followed by biochemical confirmation tests, including Gram staining and carbohydrate fermentation tests. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method. The results indicated that 8(3.1%) of the samples were positive for Listeria monocytogenes. The isolates demonstrated 100% sensitivity to Ciprofloxacin and Erythromycin, indicating their efficacy. However, the highest resistance was observed against Norfloxacin (2, 23.0%), followed by the ß-lactams (Amoxacillin and Ampiclox), as well as Streptomycin, with resistance rates reaching up to 12.5%. The study recommends implementing antibiotic stewardship programs, enhancing food safety measures, and conducting regular surveillance of L. monocytogenes in food and environmental samples. The study provides a foundational database for monitoring resistance trends in the study region, and can aid the design of epidemiological and intervention strategies in the future.

INTRODUCTION

From a chemical perspective, the term "heavy metal" is used only to describe transition metals with atomic masses exceeding 20 and specific gravities greater than 5. In biology, the term "heavy" describes a group of metals and metalloids that can be harmful to plants and animals, even at very low levels. In this context, the term "heavy metals" will be used to denote elements that have the potential to be hazardous to plants (Yadav et al., 2021). Certain heavy metals, including arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), and selenium (Se), are non-essential as they do not serve any recognised physiological role in plants. On the other hand, Co, Cu, Fe, Mn, Mo, Ni, and Zn are vital elements necessary for the regular development and metabolism of plants. When the concentration of these elements exceeds acceptable levels, it can easily result in poisoning. Heavy metal phytotoxicity can occur when enzymes stop functioning, the functional groups of important molecules are blocked, essential elements are moved or replaced, and membrane integrity is compromised. These alterations can affect various physiological processes at the cellular or molecular level (Madhu and Sadagopan, 2020).

Moreover, plants utilise a range of natural and external defence mechanisms to tolerate or neutralise the harmful effects of high concentrations of heavy metals (HMs) under stressful conditions. Consequently, certain plants can thrive, develop, and reproduce in both naturally metalliferous soils and areas contaminated with heavy metals from human activity. Most species that can withstand high levels of poisonous heavy metals, which are harmful to other plants, exhibit a behaviour known as "exclusion" (Gao et al., 2022).

They rely on tolerance and even hypertolerance methods to prevent the entry of metals into their systems. The root tissues of these plants effectively contain and detoxify the majority of heavy metals while minimising their movement to the leaves; however, the cells in the leaves remain susceptible to their harmful effects. On the other hand, several species that are very resistant to heavy metals, known as "hyperaccumulators," such as sunflower, exhibit contrasting behaviour in terms of the intake and distribution of these metals throughout the plant (Kohli et al., 2019).

The term "hyperaccumulator" was introduced to describe plants that, unlike excluder plants, actively absorb very high levels of one or more heavy metals from the soil. Furthermore, these heavy metals do not remain confined to the roots; they actively migrate up the shoot and accumulate in above-ground organs, particularly the leaves, reaching concentrations 100–1000 times higher than in non-hyperaccumulating species. In addition to their ecological and physiological significance, hyperaccumulator plants have attracted significant attention for their potential uses and practical applications. Specifically, they are being explored for their ability to remediate heavy metal-contaminated soils through phytoremediation as well as for extracting valuable metals from mineralized sites (Manara *et al.*, 2020). The term "cyanides" refers to substances that possess the C≡N group. Cyanide is a naturally occurring chemical present in many components of our natural environment. Despite its notorious toxicity, is also used in various industrial applications such as electroplating, metallurgy, organic chemical synthesis, photographic development, plastic manufacturing, ship fumigation, and certain mining procedures (Dmitry, 2021). Given the extensive applications of cyanide, it is crucial to understand its impact on plant life in the event of inadvertent introduction into an ecosystem. The early effects of cyanide exposure on plant life vary significantly across species. Excessive levels of cyanide can impede respiration and hinder a plant's capacity to uptake nutrients from the soil, potentially leading to plant mortality (Kumar *et al.*, 2016). At lower levels, cyanide can inhibit new plant growth and affect seed germination (Chaudhary and Gupta, 2012). Moreover, cyanide exhibits considerable mobility in soil, indicating its significant capacity to affect plants and other organisms rather than being immobilized by soil particles.

MATERIALS AND METHODS

Study Area

The study was conducted at the screen house of the Centre for Plant Tissue Culture and Biotechnology (CPTC-Biotech) and Centre for Advanced Science Research and Analytical Services (CASRAS) of Usmanu Danfodiyo University, Sokoto (UDUS). Sokoto State lies between latitudes 11° 30 N and 14° 00 N, and longitudes 4° 00 E and 6° 40 E, at an altitude of 351.0m above sea level (SERC, 2015). It falls within the Sudan savanna zone and is characterized by distinct wet and dry seasons that exhibit yearly variation in duration and intensity. The duration of the wet season is about three to five months, starting from May/June to August/September, with the maximum rainfall (around 600mm-700mm) recorded in July and August. Significant plant growth takes place during this period (Tsoho and Salau, 2012). The average temperature is 30ºC, with a minimum of 20ºC and a maximum of 40ºC (WWIS, 2016). The vegetation consists of scattered short trees and shrubs with dominant green cover.

Soil Sampling

The soil samples used for this study were obtained from a normal garden soil collected from the Botanical Garden of UDUS. The soil was collected at random points, homogenized and air-dried before further analysis.

Plant Materials and their Sources

Sunflower seeds were collected from matured plants growing in the Biological Garden and taken to the Herbarium of the Department of Plant Science, Faculty of Chemical and Life Sciences, UDUS for further identification. The seeds were sorted to remove soil particles, dirt, and debris before being placed in an oven (at 600C) for 2 days to break any possible dormancy. The seeds are stored in air tight containers until use. The sample was identified and authenticated, and a voucher number (UDUH/ANS/0973) was obtained for the deposited specimen.

Soil Analysis

The collected soil sample was transferred to a plastic tray for air-drying. Large clods were broken up to speed up drying, while other debris, including plant residues, was removed. After drying, the total sample was weighed, sieved through a 2 mm sieve, and clods not passing through were carefully crushed in a mortar and pestle and sieved again.

The <2 mm fraction (air-dry fine earth) is homogenized and used for the routine laboratory procedures. (Farmaha *et al.*, 2020). The soil was analyzed for heavy metals and toxins (e.g., cyanide derivatives) present before planting the seeds. The samples were analysed for physicochemical properties such as texture,

colour, pH, nitrate-nitrogen, calcium, magnesium, etc., using a soil analysis kit. Respective extraction procedures are followed for analyzing the above properties. Reagents necessary for the analysis were provided in the kit. Physico-chemical properties of the soil are measured, and heavy metal constituents and contaminants present are analysed (Farmaha *et al.*, 2020).

Solution Formulation and Spiking

Laboratory-grade Sodium cyanide (NaCN) and lead oxide (PbO_3) were dissolved separately in distilled water to make varying concentrations. For sodium cyanide, concentrations of 0, 25, 50, and 100 mg/L were prepared and used to spike the plant groups, while for lead oxide, concentrations of 0, 100, 200, and 500 mM were used. In all treatments, 100 mL of each solution was used to spike the respective plants once, and for the control groups, 100 mL of distilled water was used for each. The solution is stored in air-tight containers until use (Ghani, 2011).

Experimental Design and Treatments

A pot experiment was conducted using a Completely Randomized Design (CRD) with four treatments and six replications. Seeds were grown in plastic pots filled with 7kg of soil supplemented with cow dung (3:1). The seeds (2-3) were sown at a 1-inch depth and thinned to one plant per pot after 7 days of germination. Lead and cyanide treatments were added to the pots at one-month seedling stage. The respective plants in each group are spiked once and observed for further changes (Hinkelmann and Kempthorne, 2008). The plants were observed weekly for 2 weeks, and each week, 3 pots from each treatment were removed for measurements and other analyses.

Evaluation of Growth and Morphological Response

After each week of cyanide and lead spiking, growth parameters —specifically: number of leaves per plant, plant height (cm), root length (cm), and shoot and root fresh weight (g) —were measured using destructive sampling (3 plants were removed from each treatment). Additionally, the harvested plants were used to determine the dry weight of sunflower shoots and roots in an oven at 65 ^{0}C for 48 hours until constant weight was obtained (Krisantini *et al.*, 2017).

Determination of Leaf Spectroscopy and Pigment Indices

The leaf spectroscopy and pigment indices were measured using SpectraView CID (CI-710; model CI 710s BioScience, Inc., Felix Instruments Applied Food Science, Camas, WA 98607). The leaf was placed in the leaf clip, and the live display measurement was enabled according to the manufacturer's instructions. The spectra and pigment indices determined are: leaf senesces, total chlorophyll content (CHPL T), chlorophyll A (CHPL A), chlorophyll B (CHPL B), Anthocyanin Reflection Index (ARI 1), plant senescence reflectance index (PSRI), soil plant analysis development (SPAD), water band index (WBI), greenness (G), carotenoid reflectance index 1(CRI 1), and carotenoid content index (CCI). The measurement was repeated on the same day and time the next week (Leonard F, 2021).

Statistical analysis

The results obtained on growth, morphology, and pigment indices were analysed using two-way analysis of variance (ANOVA) in SPSS version 20 at both 5% ($p \leq 0.05$) and 1% ($p \leq 0.01$) levels of significance. The differences between means were determined using the Least Significance Difference (LSD) test.

RESULTS

Growth and Morphological Response of Sunflower

The results for plant growth parameters, including shoot length (SL), root length (RL), and leaf number, are presented in Figures 1-7. The height of plant decreased with increasing concentration of lead and cyanide. The control (0 mM) recorded the highest height (51cm in week 1and 74cm in week 2) followed by 100 mM and 200 mM, (29cm in week 1and 62cm in week 2) and (25cm in week 1 and 67cm in week 2) and 500 mM had the least value of height (24cm in week 1 and 45cm in week 2) respectively, there is statistical difference between the treatment $p < 0.05$ (Fig. 1).

In the cyanide treatment, the result for plant height shows shows no significance statistical difference $p > 0.05$, the control 0mg/L had the highest height (64cm in week 1 and 70cm in week 2), followed by 25mg/L and 50mg/L (51cm in week 1 and 50cm in week 2) and (49cm in week 1and 41cm in week 2) and 100mg/L had the least height (39cm in week 1 and 35cm in week 2) respectively (Fig. 1). The number of leaves per plant of sunflower was affected by various lead (27 in week 1 and 39 in week 2) and followed by 100 mM (25 in week 1 and 33 in week 2) and 200 mM (24 in week 1 and 32 in week 2). And subsequently, 500 mM had the least number of leaves (21 in week 1 and 31 in week 2) (Figure 2). The number of leaves per sunflower plant was affected by various concentrations of cyanide; the control (0 mg/L) had the highest number of leaves in both weeks (25 in week 1 and 28 in week 2). Followed by 25mg/L (20 in week 1 and 23 in week 2).

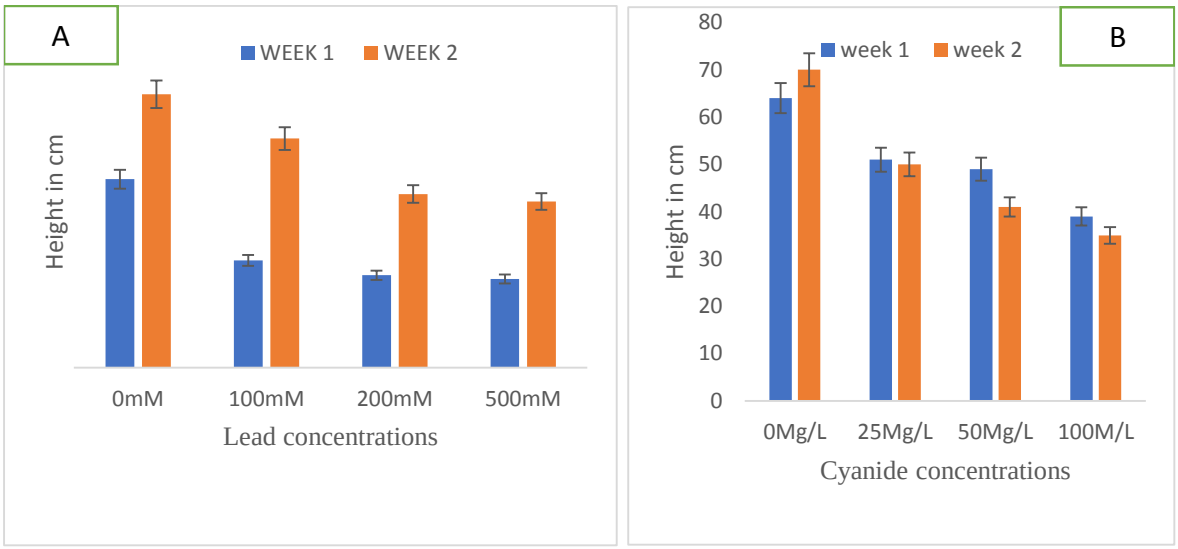

Figure 1: Plant height (cm) of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

The concentration of sunflower, the control (0mM), had the highest number of leaves in both weeks 23 in week 2, and 50 mg/L (19 in week 1 and 20 in week 2). And subsequently, 100 mg/L had the fewest leaves (17 in week 1 and 14 in week 2). There is no significance statistical difference between the treatment $p > 0.05$ (Fig 2) In addition, the result of root height in lead shows no significance statistical difference $p > 0.05$, the control 0mM had the highest height (35cm in week 1 and 29cm in week 2), followed by 100mM and 200mM (25cm in week 1and 26cm in week 2) and (27cm in week 1 and 24cm in week 2) and 500mM had the least height (25cm in week 1 and 21cm in week 2) respectively (Fig. 3).

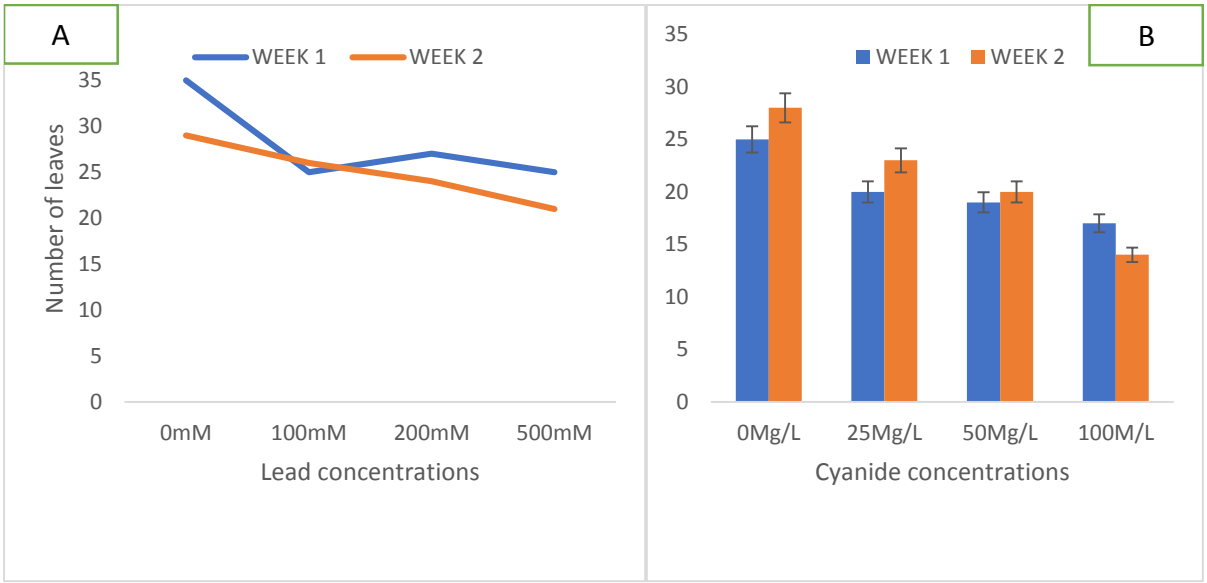

Figure 2: Number of leaves of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

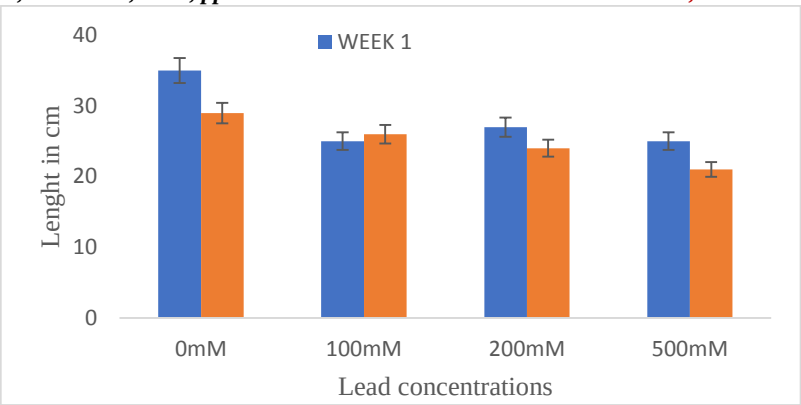

Figure 3: Root length of sunflower as affected by different concentrations of lead after 2 weeks. NB: Error bars represent % error.

Leaf Spectroscopy and Pigment Indices

In the lead, the results revealed that contaminated soils affect the survival and growth of all plants. The result with p-value 0.003 < 0.05 for PSRI implies that there is a significant difference between the effect of lead levels on PSRI of the sunflower at 5% level of significance. The results revealed that the effect of lead at 200 mM (average value of -0.80 ± 0.15 mM) is significantly lower than that of lead at 0 mM, 100 mM, and 500 mM (average values of -0.06 ± 0.01 mM, -0.07 ± 0.02 mM, and -0.07 ± 0.04 mM, respectively). The result implies that there is a significant difference between the effect of lead levels on CRI1 of the sunflower at 5% level of significance (p = 0.042). The results revealed that the effect of lead level 200 mM, with an average value of 0.30±0.15 mM, is significantly higher, followed by lead level 100 mM, 0.24±0.12 mM, and, lastly, lead levels 0 mM and 100 mM, with average values of 0.03±0.12 and 0.03±0.13, respectively, which are equally significant. However, the p-value=0.392>0.05 for WBI, p-value=0.705>0.05 for SPAD, p-value=0.699>0.05 for G, p-value=0.980>0.05 for CHPLT, p-value=0.379>0.05 for CPHLA, p-value=0.317>0.05 for CPHLB, p-value=0.994 >0.05 for ARI1 and p-value=0.790>0.05 for CCI implied that effects of lead contents at 0mM (control), 100mM, 200mM and 500mM on WBI, SPAD, G, CHPLT, CPHLA, CPHLB, ARI1 and CCI of sunflower are not statistically significant at 5 % level (Table 1). The results of comparative analyses of the effect of lead contents on a weekly basis WBI, revealed that there is a significant difference between the weekly effect of lead on WBI of the sunflower at 5% level of significance, p-value 0.043 < 0.05

Table 1: Effects of varying lead concentrations on leaf spectroscopy and pigment indices of Sunflower after 2 weeks of spiking

Group	WBI	SPAD	PSRI	G	CRI1	CHPLT	CPHLA	CPHLB	ARI1	CCI
0mM	1.14±0.08	19.7±3.53	-0.06±0.01^{b}	2.20±0.26	0.03±0.12^{c}	27.5±5.56	10.6±1.23	16.8±3.25	-0.003±0.002	6.52±1.62
100 mM	1.14±0.15	19.6±2.79	-0.07±0.02^{b}	2.33±0.27	0.03±0.13^{c}	26.8±3.97	10.3±1.38	16.4±4.31	-0.003±0.002	6.39±1.24
200 mM	1.14±0.01	18.97±4.95	-0.80±0.15^{a}	2.28±0.25	0.30±0.15^{a}	26.9±3.98	10.4±1.34	16.5±6.08	-0.002±0.003	6.27±2.29
500 mM	0.980±0.38	17.01±5.14	-0.07±0.04^{b}	2.20±0.38	0.24±0.12^{b}	24.9±4.73	9.65±1.70	15.2±3.02	-0.002±0.004	5.43±2.12
S.E	0.78	1.859	0.11	0.131	2.012	1.806	0.631	1.174	0.002	0.078
F-stat	0.164	0.473	3.509	0.482	2.947	0.980	0.820	1.065	0.124	0.350
P-value	0.392	0.705	0.003	0.699	0.042	0.337	0.379	0.317	0.994	0.790
Sig.	NS	NS	*	NS	*	NS	NS	NS	NS	NS

Legend: Values are expressed as mean ± SD, means followed by the same letter along the columns are not significantly different. * = significant at 5%, NS = Not Significant

Regarding the total chlorophyll contents (CHPLT), chlorophyll A (CHPL A), chlorophyll B (CHPL B), anthocyanin Reflection Index (ARI 1), plant senescence reflectance index (PSRI), soil plant

analysis development (SPAD), water band index (WBI), greenness (G), carotenoid reflectance index 1(CRI 1) and carotenoid content index (CCI), the results revealed that the effect of lead for week 2 with average value 1.13±0.06mM is significantly higher than that of week 1 with average values 0.83±0.54mM. The result, with p-value = 0.039 < 0.05 for SPAD, revealed a significant difference in the weekly effect of lead on SPAD of sunflower at the 5% level of significance. The results revealed that the effect of lead in week 1, with an average value of 18.2±6.423 mM, is significantly higher than that in week 2, with an average value of 15.9±4.55 mM. P-value = 0.052 < 0.10 for CRI1 indicates a significant difference in the weekly effect of lead on CRI1 of sunflower at the 10% level of significance. The results revealed that the effect of lead in week 2, with an average value of 0.214±0.03 mM, is significantly higher than that in week 1, with an average value of 0.026±0.02 mM. However, the results with p-value=0.328>0.05 for PSRI, p-value=0.533>0.05 for G, p-value=0.337>0.05 for CHPLT,p-value=0.379>0.05 for CPHLA, p-value=0.317>0.05 for CPHLB, p-value=0.179>0.05 for ARI1, and p-value=0.464>0.05 for CCI, respectively, implied that weekly effects of lead contents at weeks 1 and 2 on PSRI, G, CHPLT, CPHLA, CPHLB, ARI1, and CCI of sunflower are not statistically significant at 5 % level.

The results of comparative analyses for the effect of cyanide contents at 0 mg/L (control), 25 mg/L, 50 mg/L, and 100 mg/L on WBI, SPAD, PSRI, G, CRI1, CHPLT, CPHLA, CPHLB, ARI1, and CCI of sunflower (Table 2). SPAD implies that there is a significant difference between the effect of cyanide levels on SPAD of the sunflower at 5% level of significance with p-value 0.034 < 0.05. The results revealed that the effect of cyanide level 100 mg/L, with an average value of 31.13±26.9 mg/L, is significantly higher, followed by cyanide levels 50 mg/L, with an average value of 25.27±12.1, and lastly by 0 mg/L and 25 mg/L, with average values of 18.73±3.71 and 17.12±7.65, respectively. The result with p-value 0.026 < 0.05 for PSRI implies that there is a significant difference between the effect of cyanide levels on PSRI of the sunflower at 5% level of significance. The results revealed that the effect of cyanide level 50 mg/L, with an average value of -0.80±0.37[a] mg/L, is significantly lower, followed by cyanide levels 25 mg/L with an average value of -0.22±0.31, and lastly 0 mg/L and 100 mg/L with average values of -0.06±0.02 and -0.09±0.31, respectively. The result with p-value 0.044 < 0.05 for CRI1 implies that there is a significant difference between the effect of cyanide levels on CRI1 of the sunflower at 5% level of significance. The results revealed that the effect of cyanide at 100 mg/L (average 0.518±0.53 mg/L) is significantly higher than cyanide at 0 mg/L, 25 mg/L, and 50 mg/L (averages 0.303±0.14, 0.295±0.16, and 0.253±0.15, respectively), which is equally significant.

The p-value 0.047 < 0.05 for CHPLT implies that there is a significant difference between the effect of cyanide levels on CHPLT of sunflower at 5% level of significance. The results revealed that the effect of cyanide at 100 mg/L (average 33.5±13.1 mg/L) is significantly higher than cyanide at 0 mg/L, 25 mg/L, and 50 mg/L (averages 26.5±5.39, 25.4±6.97, and 25.0±6.87 mg/L, respectively), which are equally significant.

The result for CPHLA with p-value= 0.038 < 0.05 implies that there is a significant difference between the effect of cyanide levels on CPHLA of the sunflower at 5% level of significance. The results revealed that the effect of cyanide levels 0 mg/L and 100 mg/L, with average values 12.31±4.41 mg/L and 12.29±3.81 mg/L, is significantly higher than cyanide levels 25 mg/L and 50 mg/L, with average values 9.69±2.64 mg/L and 9.67±2.54 mg/L, respectively, which are equally significant. The result for CPHLB with p-value= 0.032 < 0.05 implies that there is a significant difference between the effect of cyanide levels on CPHLB of the sunflower at 5% level of significance. The results revealed that the effect of cyanide at 100 mg/L (average 21.08±9.32 mg/L) is significantly higher than cyanide at 0 mg/L, 25 mg/L, and 50 mg/L (average 16.12±3.29 mg/L, 15.58±4.31 mg/L, and 15.24±6.08 mg/L, respectively), which are equally significant. The result with p-value 0.025 < 0.05 for ARI1 implies that there is a significant difference between the effect of cyanide levels on ARI1 of the sunflower at 5% level of significance.

Table 2: Effects of varying cyanide concentrations on leaf spectroscopy and pigment indices of Sunflower after 2 weeks of spiking

	Parameters, Average Values (± SD) and Statistical Significance									
Group	**WBI**	**SPAD**	**PSRI**	**G**	**CRI1**	**CHPLT**	**CPHLA**	**CPHLB**	**ARI1**	**CCI**
0 mg/l	1.15±0.01	18.73±3.71^{c}	-0.06±0.02^{c}	2.44±0.69	0.303±0.14^{b}	26.5±5.39^{b}	12.31±4.41^{b}	16.12±3.25^{b}	6.04±1.61^{c}	1.14±0.01
25 mg/L	1.14±0.01	17.12±7.65^{c}	-0.22±0.31^{b}	2.12±0.48	0.295±0.16^{b}	25.4±6.97^{b}	9.69±2.64^{b}	15.58±4.31^{b}	5.74±2.58^{c}	1.14±0.15
50 mg/L	1.15±0.02	25.27±1 2.1^{b}	-0.09±0.31^{c}	2.30±0.50	0.253±0.15^{b}	25.0±6.87^{b}	9.67±2.544^{b}	15.24±6.08^{b}	11.8±1 0.9^{b}	1.14±0.11
100 mg/L	1.14±0.73	31.13±2 6.9^{a}	-0.80±0.37^{a}	2.33±0.51	0.518±0.53^{a}	33.5±1 3.1^{a}	12.29±3 .81^{a}	21.08±9 .32^{a}	35.5±7 0.4^{a}	0.98±0.38
S.E	0.15	6.255	0.245	0.012	3.655	1.474	2.448	14.564	0.078	
F-stat	0.051	3.058	0.305	1.947	2.185	2.089	2.251	3.938	1.064	
Pvalue	0.984	0.034	0.821	0.044	0.047	0.038	0.032	0.025	0.392	
Sig.	NS	*	0.047 *	NS	*	*	*	*	*	NS

Legend: Values are expressed as mean ± SD, Means followed by the same letter along the columns are not significantly different. * = significant at 5%, NS = Not Significant Total chlorophyll contents (CHPL T), chlorophyll A (CHPL A), chlorophyll B (CHPL B), anthocyanin Reflection Index (ARI 1), plant senescence reflectance index (PSRI), soil plant analysis development (SPAD), water band index (WBI), greenness (G), carotenoid reflectance index 1(CRI 1), and carotenoid content index (CCI).

The results revealed that the effect of cyanide level 100 mg/L, with an average value of 35.5±70.4 mg/L, is significantly higher, followed by cyanide levels 50 mg/L, with an average value of 11.8±10.9 mg/L, and lastly by 0 mg/L and 25 mg/L, with average values of 6.04±1.61 mg/L and 5.74±2.58 mg/L, respectively. However, the result with p-value=0.984>0.05 for WBI, p-value=0.821>0.05 for G, and p-value=0.392>0.05 for CCI implied that effects of cyanide contents at 0 mg/L (control), 25 mg/L, 50 mg/L, and 100 mg/L on WBI, G, and CCI of sunflower are not statistically significant at 5 % level.

The results of comparative analyses of the effect of cyanide contents on weekly basis (Week 1 and Week 2) on WBI, SPAD, PSRI, G, CRI1, CHPLT, CPHLA, CPHLB, ARI1 and CCI of sunflower revealed that there is significant difference between the weekly effect of cyanide on SPAD of the sunflower at 5% level of significance with p-value= 0.011 < 0.05. The results revealed that the effect of cyanide in week 2, with an average value of 40.9±38.9 mg/L, is significantly higher than that in week 1, with an average value of 21.3±2.75 mg/L. The result, with p-value = 0.031 < 0.05 for PSRI, revealed a significant difference in the weekly effect of cyanide on PSRI of sunflower at the 5% level of significance. The results revealed that the effect of cyanide in week 2, with an average value of -0.90±0.36 mg/L, is significantly lower than that in week 1, with an average value of -0.09±0.03 mg/L. The result for CRI1 revealed a significant difference in the weekly effect of cyanide on CRI1 of the sunflower at the 5% level of significance (p-value = 0.000 < 0.05). The results revealed that the effect of cyanide in week 2, with an average value of 0.73±0.07 mg/L, is significantly higher than that in week 1, with an average value of 0.03±0.02 mg/L. The result with p-value = 0.016 < 0.05 for CHPLT revealed a significant difference in the weekly effect of cyanide on CHPLT of sunflower at the 5% level of significance. The results revealed that the effect of cyanide in week 2, with an average value of 38.8±18.1 mg/L, is significantly higher than that in week 1, with an average value of 28.2±4.32 mg/L. The p-value = 0.059 < 0.1 for CPHLA indicates a significant difference in the weekly effect of cyanide on CPHLA of sunflower at the 10% level of significance. The results revealed that the effect of cyanide in week 2, with an average value of 13.82±5.18 mg/L, is significantly higher than that in week 1, with an average value of 10.76±1.59 mg/L. The result with p-value = 0.047 < 0.05 for CPHLB revealed a significant difference in the weekly effect of cyanide on CPHLB of sunflower at the 5% level of significance.

The results revealed that the effect of cyanide in week 2, with an average value of 24.86±12.9 mg/L, is significantly higher than that in week 1, with an average value of 17.21±2.78 mg/L.

The result with p-value = 0.037 < 0.05 for ARI1 revealed a significant difference in the weekly effect of cyanide on ARI1 of sunflower at the 5% level of significance. The results revealed that the effect of cyanide in week 2, with an average value of -0.77±0.12 mg/L, is significantly higher than that in week 1, with an average value of -0.24±0.03 mg/L. The result for CCI revealed that there is a significant difference between the weekly effect of cyanide on the CCI of the sunflower at 5% level of significance, with a p-value of 0.011 < 0.05. The results revealed that the effect of cyanide in week 2, with an average value of 63.8±19.9 mg/L, is significantly higher than that in week 1, with an average value of 7.21±1.46 mg/L. However, the result with p-value=0.984>0.05 for WBI and p-value=0.880>0.05 for G, respectively, implied that the weekly effects of cyanide contents at weeks 1 and 2 on WBI and G of sunflower are not statistically significant at 5 % level.

Plant Biomass of Sunflower

The result on the effect of lead on biomass of sunflower in shoot fresh water (SFW) shows that there is no statistical significance difference between the treatment p> 0.05, control (0mM) has the highest weight (week 1 86.8g and week 2 73.0g) followed by 100mM and 200mM, (77.1g in week 1and 42.2g in week 2) and (67.1g in week 1 and 40g in week 2) and 500mM had the least weight (50.7g in week 1 and 38.3g in week 2) respectively (Fig 4). The result on the effect of cyanide on biomass of sunflower in shoot fresh water (SFW) shows no significance statistical difference p > 0.05, the control 0 mg/L had the highest weight (46.2g in week 1 and 50.8g in week 2), followed by 25 mg/L and 50 mg/L (36.1g in week 1 and 30.1g in week 2) and (25.9g in week 1and 28.1g in week 2) and 100 mg/L had the least height (22.1g in week 1 and 22.7g in week 2) respectively (Fig.4).

Furthermore, result on shoot dry weight (SDW) in lead shows that there is no statistical significance difference p> 0.05, the control 0mM shows the highest dry matter 13.2g in week 1and 13.3g in week 2 followed by 100mM and 200mM 11.0g in week 1 and 9.8g in week 2, 10.9g in week 1and 9.2g in week 2 and 500mM had the least weight 10.0g in week 1 and 7.0g in week 2 respectively, (Fig. 5). Result on shoot dry weight (SDW) shows that there is no statistical significance difference p> 0.05, the control 0 mg/L shows the highest dry matter 11.6g in week 1and 12.5g in week 2 followed by 25 mg/L and 50 mg/L 8.5g in week 1 and 5.8g in week 2, 7.6g in week 1and 4.2g in week 2 respectively and 100 mg/L had the least weight 3.6g in week 1 and 3.1g in week 2 respectively, (Fig.5).The result on the effect of lead on biomass of sunflower in root fresh weight (RFW) shows that there is no statistical significance

difference p> 0.05, control 0mM has the highest weight (week 1 14.6g and week 2 13.4g) followed by 100mM and 200mM, (11.0g in week 1and 9.8g in week 2) and (9.9g in week 1 and 9.2g in week 2) and 500mM had the least weight (9.0g in week 1 and 7.8g in week 2) respectively, (Fig. 6).

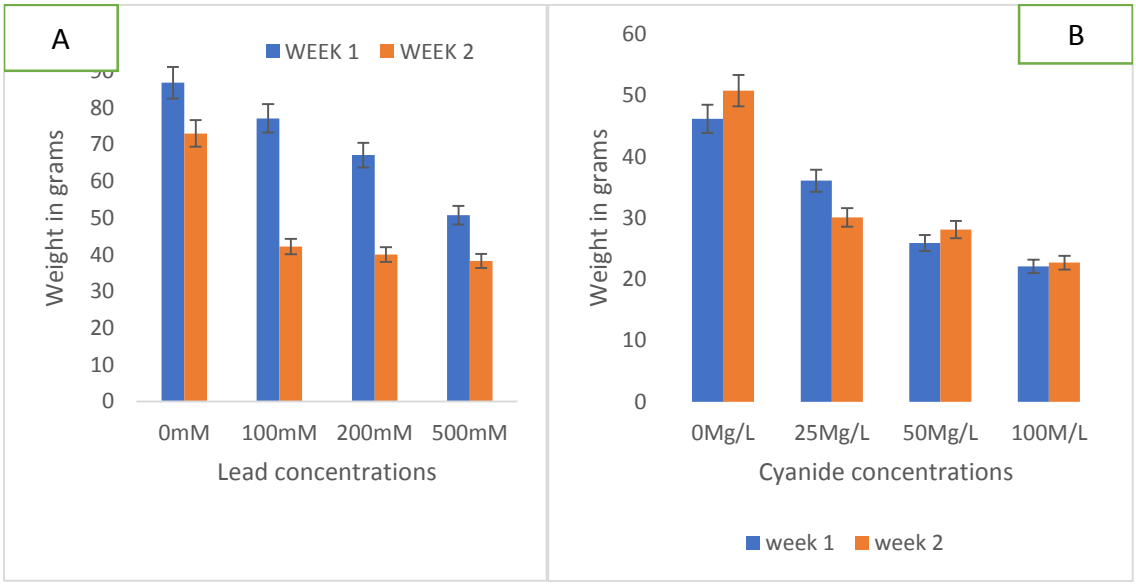

Figure 4: Fresh weight of the shoot of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

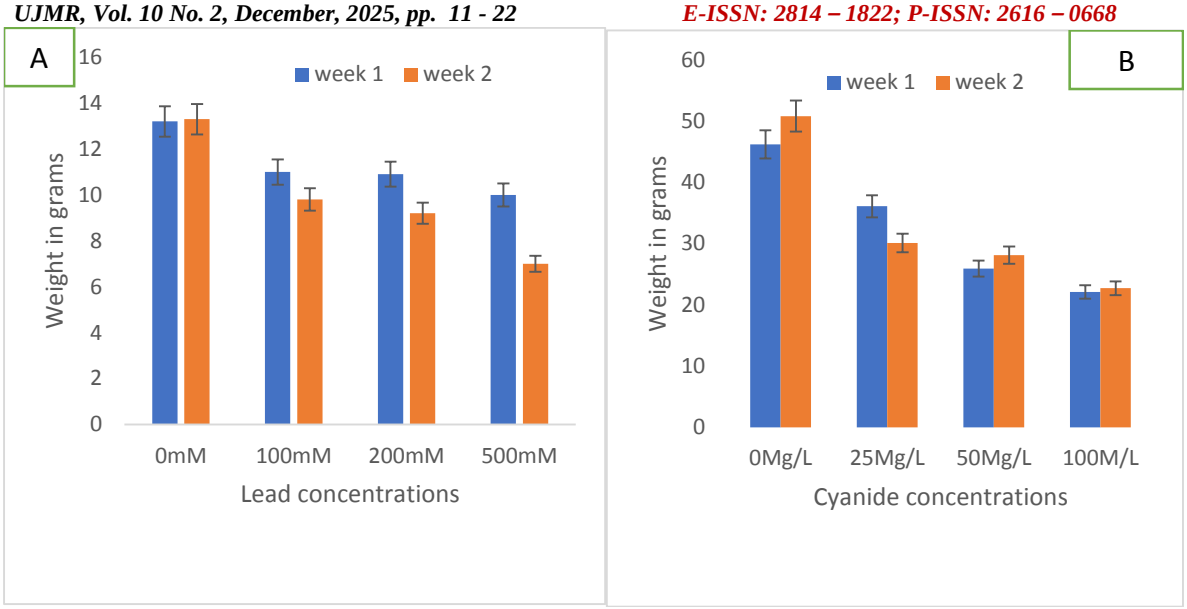

Figure 5: Dry weight of the shoot of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

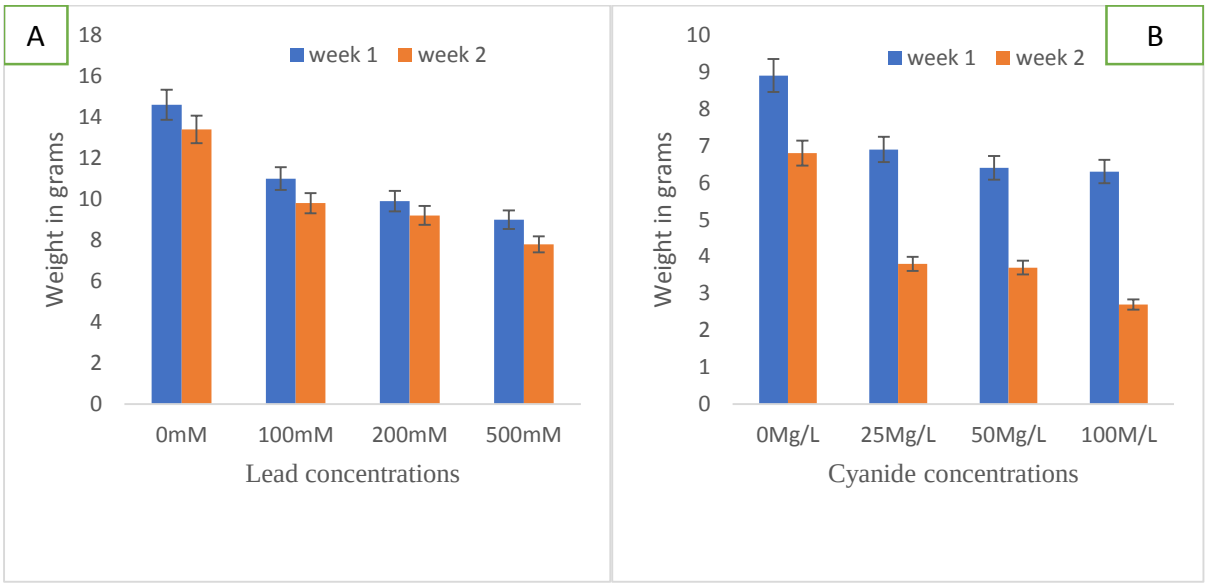

Figure 6: Root Fresh weight of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

The result on the effect of cyanide on biomass of sunflower in root fresh weight (RFW) shows that there is statistical significance difference $p < 0.05$, control 0 mg/L has the highest weight (week 1 8.9g and week 2 6.8g) followed by 25 mg/L and 50 mg/L, (6.9g in week 1and 3.8g in week 2) and (6.4g in week 1 and 3.7g in week 2) and 100 mg/L had the least weight (6.3g in week 1 and 2.7g in week 2) respectively, (Fig.6). Root dry weight (RDW) in lead shows that there is no statistical significance difference $p > 0.05$, the control 0mM shows the highest dry matter 3.5g in week 1and 4.2g in week 2 followed by 100mM and 200mM 2.5g in week 1 and 2.3g in week 2, 2.3g in week 1and 2.3g in week 2 and 500mM had the least weight 1.1g in week 1 and 1.7g in week 2 respectively, (Fig. 7). Root dry weight (RDW) in cyanide shows that there is no statistical significance difference $p > 0.05$, the control 0 mg/L shows the highest dry matter 1.7g in week 1and 2.3g in week 2 followed by 25 mg/L and 50 mg/L, 1.4g in week 1 and 1.3g in week 2, 1.1g in week 1and 1.1g in week 2 and 100 mg/L had the least weight 0.4g in week 1 and 0.5g in week 2 respectively, (Fig.7).

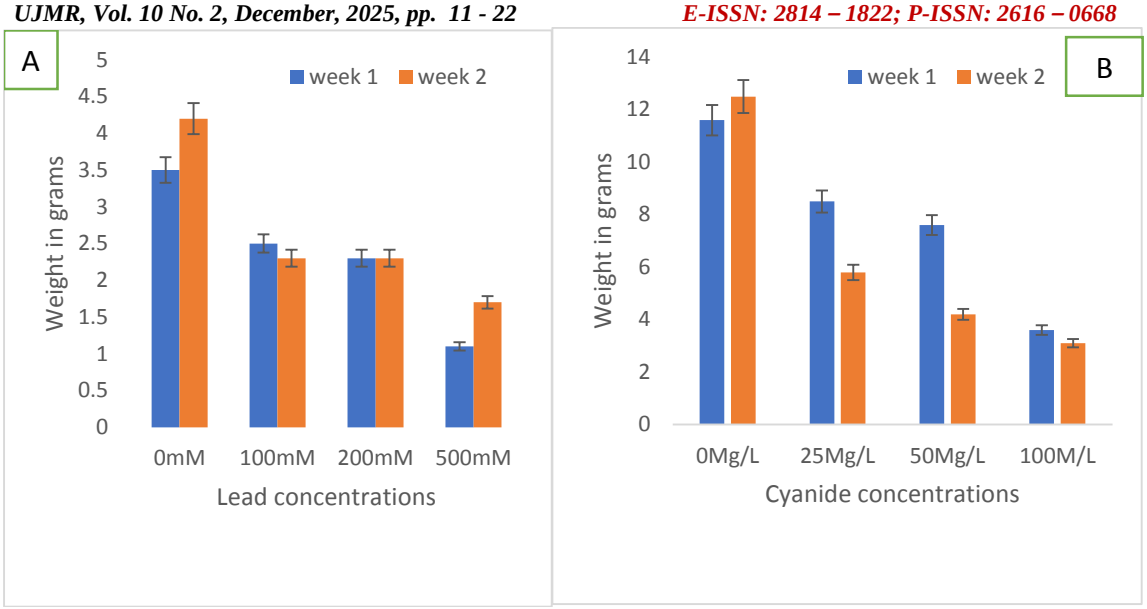

Figure 7: Root dry weight of sunflower (a) as affected by different concentrations of lead after 2 weeks and (b) as affected by different concentrations of cyanide after 2 weeks. NB: Error bars represent % error.

DISCUSSION

Plant growth, physiology, and antioxidant enzyme activities are susceptible to metal toxicity, as evidenced by cell injury and plant tolerance to metal stress (Aslam et al., 2021). In the present work, plant height was significantly affected when sunflowers were exposed to lead and cyanide contamination. Soares *et al*. (2001) reported that higher concentrations of heavy metals have been reported to retard cell division and differentiation, reduce elongation, and affect plant growth and development. The root and shoot length of the treated seedlings were also found to be considerably reduced at all levels of the heavy metals applied, except the control, where root length was more or less comparable with treated seedlings. The adverse effects on root and shoot growth were much more pronounced at the highest concentrations of cyanide (100 mg/L) and lead (500 mM), which may be one aspect of the role of metabolic inhibitors in the overall phenomenon of plant growth. Many studies have reported the inhibitory effect of various heavy metals, including Cd and Pb, on plant growth (Sharma and Dubey 2005; Mishra *et al*., 2006).

Moreover, this study reveals that heavy metals affect leaf number, with the highest concentrations of lead and cyanide associated with the lowest number of leaves in both weeks. In a previous study, pathological changes at the ultrastructural level were confirmed in other plant species upon exposure to heavy metals (Goyal *et al*., 2020). Leaves are considered as one of the most important plant organs because of their role in capturing light and making food by the process of photosynthesis. Farooqi *et al*. (2009) conducted a study on soybean that indicated that heavy metal toxicity induced histological changes in leaves, resulting in thin leaf blades, minification of the xylem and phloem in the vascular bundles, and reduced xylem vessel diameter. At the same time, all these damages could disrupt many plant activities, including the antioxidative system, photosynthesis, respiration, mineral nutrition, membrane structure and properties, and gene expression.

Furthermore, the observed negative impacts on sunflower physiology confirmed that the presence of lead and cyanide concentrations in the soil above tolerable limits poses a threat to plant physiological machinery, resulting in chlorosis, necrosis, rolling, and twisting of leaves, eventually impeding the plant's health (Shabaan *et al*.,2020; Ahmed *et al*.,2022). Additionally, the results of this study are similar to those of previous studies, which reported severe adverse effects on plant growth and physiology under lead stress.

Carotenoids serve as antioxidants against free radicals and photochemical damage (Sengar *et al*., 2008). The results of the current study reveal no significant ($p<0.05$) change in chlorophyll a and b, and carotenoids, indicating that the sunflower plant may have a high ability to tolerate lead stress. The deterioration of chlorophyll synthesis due to lead toxicity resulted in chlorotic leaves, changed ratios of chlorophyll a and b (Viehweger and Geipel, 2010), and photosynthetic activity. Exposure to cyanide and lead significantly decreased dry matter in experimental plants.

The considerable reduction in plant dry matter yields under the influence of high concentrations of heavy metals agrees with the significant decrease in dry biomass of Albizzia lebbeck reported under the toxic effects of lead and cadmium (Farooqi et al., 2009). The growth-development, fresh-dry biomass, and growth tolerance index of root, shoot, and leaves were negatively altered by increasing lead and cyanide concentrations in sunflower. Similar results were obtained in other studies at the calculated lead concentration: root, shoot, and leaf growth, and fresh and dry biomass were critically reduced in Zea mays (Wang et al., 2021). The weight of the shoot was significantly higher than that of the roots. Other authors (Ashraf *et al.*,2019; Alaboudi *et al.*,2018) found reductions of dry matter production in many plant species as a function of the application of increasing doses of heavy metals in experiments with soil and nutrient solution.

CONCLUSION

This study confirmed the tolerance limits and mechanisms of heavy metal tolerance in sunflower as assessed under three levels of lead and cyanide exposure. All the growth parameters were affected at the different concentrations tested. The results from this study would certainly help identify strategies to alleviate sunflower stress from heavy metals towards exploring and enhancing the phytoremediation potential of the plant.

RECOMMENDATIONS

1. The levels of potentially toxic heavy metals and metalloids in water, sediments, soils, and the resident biota should be assessed and regularly monitored.
2. The public should be educated about the harmful effects of toxic heavy metals on human health and the environment.
3. Sunflowers can be effectively used in phytoremediation to remediate heavy metals contaminated soils around metal smelting factories, mining sites, etc.

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