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EXPLORING THE ROLE OF EPIGENETIC MODIFICATIONS IN PLANT RESPONSES TO HEAVY METAL STRESS

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ABSTRACT

The study aimed to examine the epigenetic and physiological mechanisms in Arabidopsis thaliana and Oryza sativa under cadmium (Cd) and lead (Pb) stress, an increasing concern due to heavy metal pollution, and to investigate how plants can withstand these stresses. The experiment investigated changes in DNA methylation, histone modifications, and gene expression in response to metal stress. Data were obtained through bisulfite sequencing and the ChIP-qPCR test (chromatin immunoprecipitation with a QIAcube automated nucleic acid purification system and real-time PCR system), and the gene expression was observed through RT-qPCR. Physiological parameters, including biomass, chlorophyll content, electrolyte leakage, and metal accumulation, were determined using spectrophotometry and atomic absorption spectroscopy. ANOVA and Pearson correlation coefficients were used to test significance and relationships. The results revealed that the exposure to Cd and Pb caused widespread reprogramming of the epigenome: in *Arabidopsis*, MET1 concentrations dropped by 45% during Cd stress, and ROS1 concentrations rose by 60%, leading to a 20% decrease in global methylation. Promoters of metal homeostasis genes were enriched for histone marks H3K4me3 and H3K27me3 and were associated with a two- to threefold increase in detoxification gene expression. Physiologically, there was a decrease in growth of up to 30% and a 50% growth in root metallic content of treated plants. The findings indicate that epigenetic alterations are important in mediating plant responses to heavy metal stress and could serve as biomarkers for the development of tolerant crop genotypes and for enhancing phytoremediation.

***Keywords*:** Epigenetics, Heavy metal stress, DNA methylation, Histone modification, Phytoremediation.

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1. INTRODUCTION

Agricultural soils contain heavy metals that are very harmful to crop yields, food security, and ecosystem sustainability. The key sources are the dumping of industries, mining, excessive use of phosphate fertilizers, and wastewater irrigation (Cui et al., 2023; Rashid et al., 2023). These unnecessary heavy metals are also toxic to both animals and plants, interfering with cellular activities and causing adverse effects. Heavy metals disrupt photosynthesis and nutrient uptake, causing oxidative stress and retarding plant growth. They have a biological persistence and build up in plant tissues and are transferred to the food chain, which has health hazards for both human beings and animals (Okereafor et al., 2020; Kumar et al., 2020; Alengebawy et al., 2021; Mansoor et al., 2023; Angon et al., 2024). Research on plant responses to heavy metal stress is essential for developing sustainable practices that enhance resistance and minimize metal levels in plant edible tissues.

Plants developed physiological, biochemical, and molecular mechanisms to cope with heavy metal stress. Physiologically, heavy metal exposure causes retarded growth, decreased chlorophyll content, and electrolyte leakage, indicating membrane destabilization and cell damage (Shahid et al., 2014; Rahman et al., 2022; Afzal et al., 2024; Jomová et al., 2024; Boonkhao et al., 2025). Plants have a complex antioxidant defense system that minimizes oxidative stress by producing enzymatic antioxidants such as superoxide dismutase, catalase, and peroxidases, and non-enzymatic antioxidants such as glutathione and ascorbate (Riyazuddin et al., 2021; Ansari et al., 2024; Bashir et al., 2025). They also synthesize chelating organic acids, phytochelatins, and metallothioneins, which bind complex heavy metals and woody matter, sequestering them and making them less toxic. Plants regulate the uptake of heavy metals, sequestration (Raza et al., 2021), and detoxification on the molecular level through regulating transporter genes HMA2 and HMA3 (Heavy Metal ATPases) (Riyazuddin et al., 2021; Tao & Lu, 2022; Skuza et al., 2022), PCS1 (phytochelatin synthase), and NRAMPs (Natural Resistance-Associated Macrophage

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Proteins) (Nosek et al., 2020; Tian et al., 2021). Epigenetics is also major in modifying the response of plants to heavy metal stress.

Epigenetics is defined as hereditary, reversible alterations in gene expression that are not associated with changes in the fundamental DNA sequence. Such changes include alterations in DNA methylation, histone modifications, and gene silencing by small RNAs (Li, 2020). Plants can adapt to stressors such as heavy metal toxicity by dynamically adjusting their gene expression patterns. The silencing of genes in promoter regions results from DNA methylation, which entails the predominant insertion of methyl groups onto cytosine residues (Gallo-Franco et al., 2020; Sun et al., 2022; Fasani et al., 2023). Modifications of the histones, including the acetylation or methylation of the separate histone residues, influence the chromatin organization and accessibility of DNA to transcription. The histone modification process is another mechanism that alters the interaction between histones and DNA to influence gene expression. One type of chemical modification is the H3K4me3, which is associated with gene reactivation and suppression (Liu et al., 2022; Jin et al., 2025). Furthermore, Ibragić et al. (2025) reported that epigenetic modification can alter chromatin structure and gene activity without altering the underlying DNA sequence.

Epigenetic marks in plants form a memory that enables them to react better to a repeat of stresses (Chmielowska-Bąk et al., 2023). Plants undergo dynamic epigenetic reprogramming under heavy metal stress, and this may be transgenerational. This process enables plants to acquire adaptive properties, making them more resistant to polluted environments (Dutta et al., 2018; Akhter et al., 2021; Greco et al., 2025). Heavy metals such as cadmium cause hypomethylation of plant species such as *Arabidopsis thaliana* and rice, which involves the mediating effects on the regulation of detoxification and stress response genes. Hypermethylation can repress vulnerable/destructive genes and permit an open chromatin structure of defense-related genes (Feng et al., 2016; Sun et al., 2021; Gao et al., 2025). The stress of heavy metals induces specific changes in histones that dictate the expression of plant genes. Stress with cadmium leads to an increase in H3K4me3 at promoters of metal carrier genes and a reduction in repressive markers that promote expression, facilitating heavy metal metabolism by chelation and detoxification (Feng et al., 2016; Qu & Zheng, 2024; Gocek-Szczurtek et al., 2025). These modifications play a role in broader chromatin remodeling, which enhances plant adaptation to heavy metal stress.

Plants are more tolerant to heavy metals through the production of complex control systems by changes in their DNA and organs, as a result of epigenetic modifications and physiological responses. Stress on cadmium downregulates the DNA methyltransferase gene MET1, leading to hypermethylation of DNA and the expression of stress-response genes. Increased expression of histone demethylases, including JMJ705, enhances detoxification pathways (Pan et al., 2024). Kakoulidou et al. (2021) and Ali et al. (2022) documented that biotechnological methods are seeking to identify epigenetic regulator manipulation to promote phytoremediation and crop quality. Targeted modifications of DNA methyltransferases or histone-modifying enzymes could be used to reprogram stress-responsive gene networks, thereby reducing metal uptake or enhancing detoxification in plants. When integrated with traditional breeding and modern genetic engineering, these approaches could promote sustainable agricultural systems capable of thriving under conditions of environmental pollution. Physiological defects such as reduced biomass, chlorophyll content, and electrolyte leakage are associated with epigenetic changes. According to scientists, epigenetic modulations are functional processes that enhance stress tolerance and crop yield and durability. Plant epigenetics can be transgenerational, benefiting adaptively but leaving the genetic code unaltered (Fasani et al., 2023). This has dire implications for the agricultural sector, especially in polluted environments, where crops may develop resistance through epigenetic memory.

Biotechnological tools have focused on epigenetic manipulation to enhance phytoremediation and the quality of crops. A stress-responsive network can be reprogrammed to reduce metal uptake or enhance detoxification by altering DNA methyltransferases or histone modifiers. This along with traditional breeding and genetic modification may result in sustainable farming technologies that will be able to deal with environmental pollution. Nonetheless, the literature of epigenetic response of plants to heavy metal stress is comparatively small relative to other types of abiotic stressors. The existing body of understanding is premised on information about the correlations between molecular changes of epigenetic modifications and functional reactions to stress, especially in *Arabidopsis* thaliana.

The problem of heavy metal pollution is serious to agriculture and food security and requires innovative and sustainable solutions. Epigenetic changes facilitate tolerance, detoxification, and sequestration of the plants to heavy metals by modifying DNA, histone, and silencing of the genes by small RNA-induced changes (Iqbal et al., 2024). The resilience of crops could be improved by developing action and interaction mechanisms between the epigenetic processes and plant responses to stress. Biotechnological intervention of epigenetic control factors can enable scientists to develop crops that are able to survive in contaminated soils, reduce the amount of metals in food that one consumes, and enhance phytoremediation.

Epigenetics and epigenomics play an essential role in understanding plants' mechanisms of tolerance to heavy metal stress and hold promise for enhancing agriculture and environmental management. Future studies will assist in translating molecular knowledge into practical implementation in the field, thereby creating strong crop

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Open Access Journal

production systems capable of surviving soil pollution. The objectives of the study were to find the dynamism of the DNA methylation and histone modification variables, physiological alterations in biomass and chlorophyll content, electrolyte leakage, and metal deposition, and also to establish biomarkers of heavy metal tolerance. Measuring physiological changes in biomass and chlorophyll content, electrolyte leakage, and metal accumulation and comparing the epigenetic and physiological response of the two plant species were also the objectives of the research. The study will be useful in establishing strong crop production systems that will resist soil pollution and enhance agriculture.

2. MATERIALS AND METHODS

This study aimed to describe how epigenetic changes regulate plant responses to heavy metal stress by integrating molecular, biochemical, and physiological approaches. Two plant species, namely Arabidopsis thaliana (a model organism) and the regular crop species Oryza sativa (rice), were chosen for study. Some grains of *Arabidopsis thaliana* (Col-0) and *Oryza sativa* (cv. IR64) were surface-sterilized through immersion in 70% ethanol over 2 minutes and then reduced in a 10% bleach solution containing some drops of Tween-20. Their seeds were then rinsed thoroughly with sterile distilled water to remove all sterilizing agents. The petri dishes were placed with the seeds on moist filter paper and germinated at 22°C with continuous light in three days. Three weeks later, the seedlings were moved to hydroponic culture in half-strength Hoagland nutrient solution. The treatment with heavy metal was done using cadmium chloride ($CdCl_2$ 200 mg/kg) and lead nitrate [$Pb(NO_3)_2$ 500 mg/kg]. Control plants were kept in a non-metallic nutrient solution. Sampling was performed at six time points: 0, 6, 12, 24, 72 and 168 hours after treatment, with five biological replicates per condition.

2.1. Rationale for these Doses

The selected cadmium chloride concentration (200 mg/kg) and lead nitrate concentration (500 mg/kg) were established on previous research that determined the sublethal but stress-inducing concentration of heavy metals in plants. These doses were selected to cause a significant stress response, including physiological, biochemical, and epigenetic alterations, but not result in immediate toxicity or plant death. This enabled the study to explore the mechanisms of stress tolerance and epigenetic regulation at controlled conditions of heavy metal exposure.

2.2. Preparation of Treatment Solutions

The solutions were made by initially preparing stock solutions of cadmium chloride ($CdCl_2$) and lead nitrate [$Pb(NO_3)_2$] by dissolving the right quantities of both chemicals in distilled water. Half-strength Hoagland nutrient solution was then added to these stock solutions to obtain the required different concentrations of cadmium chloride (200 mg/kg) and lead nitrate (500 mg/kg). The nutrient solutions with heavy metals were then poured into the hydroponic setup with the seedlings to ensure the same level of exposure. The precise volumes of the stock solutions to achieve the desired concentrations in the nutrient solution were calculated, and the stock solutions were mixed and aerated to ensure uniform distribution of the heavy metals throughout the solution.

2.3. Application of Treatment

The treatments were used by placing the prepared nutrient solutions that contained heavy metals in the hydroponic system that contained the seedlings. The solutions were given at a constant frequency after every few days so as to keep the levels constant. The treatment time lasted for 168 hours, with sampling readings taken at specified intervals of 0, 6, 12, 24, 72, and 168 hours after treatment. The plants were exposed to heavy metals throughout this period to evaluate their impact on plant growth and physiology.

2.4. Epigenetic and Molecular Studies

2.4.1. DNA Methylation: The genomic DNA was purified from the leaf and root tissues according to a CTAB-based procedure. A DNA methylation ELISA-based 5-methylcytosine (5-mC) assay kit was used to quantify global DNA methylation (Patriota et al., 2024). Following the step of determining the absorbance at 450 nm, the degree of methylation was determined as a percentage of methylated cytosines compared to total cytosines, which provided the high-throughput data on the methylation in addition to time (Table 1).

2.4.2. Histone Modifications: The chromatin immunoprecipitation (ChIP) and qPCR (ChIP-qPCR) were used to measure the profile of histone modification (Moravčíková & Žiarovská, 2023). The cross-linking of fresh tissues was made with 1% formaldehyde; chromatin was extracted and sonicated to produce a 200-500 bp size. Antibodies against the H3K4me3 (activating mark) and H3K27me3 (repressive mark) were employed in immunoprecipitation. Amplification of purified DNA was performed using promoter-region-specific primers for major genes, including HMA2, PCS1, and NRAMP. Fold change was computed in terms of enrichment in relation to input DNA (Table 2).

2.4.3. Gene Expression: Total RNA was extracted by using TRIzol, and then DNase I treatment was applied. The

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integrity of the RNA was checked by gel electrophoresis, and concentration was assessed spectrophotometrically. cDNA was synthesized using reverse transcriptase, and RT-qPCR was done to determine the expression of the genes such as MET1, ROS1, JMJ705, and heavy metal transporter genes (Auler et al., 2017). The expression levels were brought to control (actin and ubiquitin) levels and then computed with the fold change method (2^−ΔΔCt method). The results were presented as fold changes relative to controls (Table 3).

Table 1: Global DNA Methylation Levels (5-mC% %) over time in two species exposed to Cd or Pb

Species	Treatment	Metal Conc. (mg/kg)	Time (h)	Global 5-mC (%)	% Change vs t=0	Statistical Difference
Arabidopsis thaliana	Control	0	0	23.4 ± 1.2	—	A
A. thaliana	Control	0	24	23.6 ± 1.1	+0.9%	A
A. thaliana	Control	0	72	23.5 ± 1.3	+0.4%	A
A. thaliana	Control	0	168	23.3 ± 1.2	−0.4%	A
A. thaliana	Cd	200	0	23.5 ± 1.1	—	A
A. thaliana	Cd	200	24	20.8 ± 1.4	−11.5%	B
A. thaliana	Cd	200	72	17.6 ± 1.3	−25.1%	C
A. thaliana	Cd	200	168	15.1 ± 1.1	−35.7%	D
A. thaliana	Pb	500	0	23.6 ± 1.2	—	A
A. thaliana	Pb	500	24	21.4 ± 1.5	−9.3%	B
A. thaliana	Pb	500	72	19.2 ± 1.6	−18.6%	C
A. thaliana	Pb	500	168	17.6 ± 1.8	−25.4%	Cd
Oryza sativa	Control	0	0	26.7 ± 1.0	—	A
O. sativa	Control	0	24	26.8 ± 1.2	+0.4%	A
O. sativa	Control	0	72	26.6 ± 1.1	−0.4%	A
O. sativa	Control	0	168	26.9 ± 1.0	+0.7%	A
O. sativa	Cd	200	0	26.6 ± 1.0	—	A
O. sativa	Cd	200	24	24.9 ± 1.3	−6.4%	B
O. sativa	Cd	200	72	22.8 ± 1.4	−14.3%	C
O. sativa	Cd	200	168	20.9 ± 1.5	−21.4%	D
O. sativa	Pb	500	0	26.8 ± 1.1	—	A
O. sativa	Pb	500	24	25.7 ± 1.2	−4.1%	Ab
O. sativa	Pb	500	72	24.1 ± 1.4	−10.1%	Bc
O. sativa	Pb	500	168	22.2 ± 1.7	−17.2%	C

Mean ± SD sharing the same letter are not significantly different at P<0.05. n = 6 per timepoint.

Table 2: Selected Histone Modification Changes at Stress-Responsive Genes (Relative Enrichment, ChIP qPCR fold change vs control)

Histone Mark	Gene (Function)	Species	Treatment	Metal Conc. (mg/kg)	N	Fold Change (Mean ± SD)	Wilcoxon p-value
H3K4me3 (activating)	HMA2 (metal transporter)	A. thaliana	Control	0	4	1.00 ± 0.05	—
H3K4me3	HMA2	A. thaliana	Cd	200	4	1.85 ± 0.20	0.008
H3K4me3	HMA2	A. thaliana	Pb	500	4	1.42 ± 0.18	0.045
H3K27me3 (repressive)	PCS1 (phytochelatin synthase)	O. sativa	Control	0	4	1.00 ± 0.04	—
H3K27me3	PCS1	O. sativa	Cd	200	4	0.58 ± 0.07	0.003
H3K27me3	PCS1	O. sativa	Pb	500	4	0.75 ± 0.10	0.021
H3K9ac (active)	MT2A (metallothionein)	A. thaliana	Control	0	4	1.00 ± 0.06	—
H3K9ac	MT2A	A. thaliana	Cd	200	4	2.10 ± 0.25	0.002
H3K9ac	MT2A	A. thaliana	Pb	500	4	1.60 ± 0.20	0.019

Table 3: Expression Fold Change of Epigenetic Regulator Genes (RT-qPCR: $\log_2$ fold change vs control)

Gene	Function	Species	Treatment	Metal Conc. (mg/kg)	$\log_2$ FC Mean ± SD	Adjusted p-value (FDR)
MET1	DNA methyltransferase	*A. thaliana*	Cd	200	-1.8 ± 0.4	0.002
CMT3	CHG methyltransferase	*A. thaliana*	Cd	200	-0.9 ± 0.3	0.030
ROS1	DNA demethylase	*A. thaliana*	Cd	200	+1.5 ± 0.5	0.010
SUVH4/KYP	H3K9 methyltransferase	*O. sativa*	Pb	500	+0.3 ± 0.2	0.28
JMJ705	H3K27 demethylase	*O. sativa*	Cd	200	+1.9 ± 0.6	0.001
HDA6	Histone deacetylase	*A. thaliana*	Pb	500	+0.2 ± 0.15	0.36
DCL3	siRNA biogenesis	*O. sativa*	Cd	200	−1.2 ± 0.35	0.015

2.5. Physiological and Biochemical Measures

2.5.1. Growth and Stress Measures: Biomass (fresh and dry weight), chlorophyll content, and electrolyte leakage were evaluated as a method of measuring physiological alterations. Conductivity tests were conducted to determine electrolyte loss. The metal concentration in tissues was determined using atomic absorption spectroscopy (AAS). The extracted results for such parameters, which prove the correlation between molecular modification and stress tolerance, are provided in Table 4.

2.5.2. Experimental Design and Statistical Analysis: To strengthen the data, the experimental design included five biological replicates per treatment and time. Data analysis was done using R and SPSS. ANOVA (2-tailed) was used to compare the treatment (cadmium, lead, control) and time effect on both molecular and physiological parameters, and Tukey post hoc (P≤0.05) was applied. The relations between DNA methylation, histone

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modifications, expression of a gene, and physiological characteristics were tested with the assistance of correlation analyses (Pearson correlation coefficient). To identify molecular signatures associated with tolerance or susceptibility, principal component analysis (PCA) was performed to identify significant epigenetic marks that distinguished the plants' responses.

2.6. Data Processing

Raw data from ELISA, ChIP-qPCR, RT-qPCR, and biochemical assays were converted to standard numbers. In DNA methylation, absorbance was measured against standards to determine the percentage methylation. The enrichment of histone modification was assessed as a fold change relative to input DNA. Gene expression changes were determined using the 2^−ΔΔCt method, in which Ct values for target genes were normalized to those of housekeeping genes and expressed as fold changes relative to untreated controls. Replicates were averaged regarding physiological variables such as chlorophyll content, biomass, electrolyte leakage, and metal accumulation.

This type of molecular, epigenetic, and physiological study is an effective approach to understanding the impact of heavy-metal stress on plant epigenetics and responses. The findings revealed mechanistic associations between epigenetic modifications and plant resistance, thereby conveying facts regarding the creation of stress-resistant and phytoremediation-resistant crops.

2.7. Heavy Metal Treatments and Sampling

Plants aged three weeks were treated with 200 mg/kg $CdCl_2$ and 500 mg/kg $Pb(NO_3)_2$. Metals were absent in the control plants. The treatment durations were at 0, 6, 12, 24, 72, and 168 hours. All treatments were done 5 times biologically. At each time point, samples of the leaves and roots were collected, frozen in liquid nitrogen, and stored at -80°C.

***2.7.1. DNA Extraction and Global Methylation Assay*:** The CTAB procedure (Doyle & Doyle, 1987) was used to isolate the genomic DNA. DNA quality and DNA concentration were determined spectrophotometrically. According to the manufacturer, the quantity of global methylation was measured using a 5-mC assay kit with an ELISA protocol (Zymo Research). Absorbance was measured at 450 nm, and the percentage of methylation relative to the control samples was calculated.

2.7.2. Chromatin Immunoprecipitation/Quantitative Polymerase Chain Reaction (ChIP-qPCR): The tissues were cross-linked by putting them in a vacuum in 1% formaldehyde and left to incubate for 10 minutes. The cross-linking was quenched with 0.125 M glycine. To purify and quantify the DNA, chromatin was purified, sonicated (to acquire pieces of sheared chromatin of between 200-500 bp), and immunoprecipitated with antibodies against H3K4me3 and H3K27me3 (Abcam). qPCR of purified DNA using HMA2, PCS1, and MT2A promoter primers was used to purify the DNA. Determination was done relative to input DNA.

2.7.3. Gene Expression Analysis: TRIzol reagent (Invitrogen) was applied to isolate the total RNA, and the additional DNA was removed with the DNase I solution (Thermo Fisher). The cDNA was synthesized with the help of the SuperScript IV kit (Thermo Fisher). This RT-qPCR was performed on a CFX96 Real-Time PCR system that used SYBR Green Master Mix (Bio-Rad). The amounts of the expression of MET1, ROS1, JMJ705, HMA2, PCS1, and MT2A were normalized by the actin (in the case of *A. thaliana*) or ubiquitin (in the case of *O. sativa*). The 2^−ΔΔCt method was used to carry out relative quantification.

2.7.4. Physiological and Biochemical Measures: To obtain physical chemistry measurements, the fresh weight was considered as the biomass. Chlorophyll content was measured with the help of the SPAD-502 meter (Minolta). Electrolyte leakage was assessed by measuring the conductivity of leaf discs in deionized water using a conductance meter. The level of heavy metals was determined by digesting the plant tissues in nitric acid, and the quantification was carried out using an atomic absorption spectrophotometer (PerkinElmer AA). The results were subjected to statistical tests using R, with two-way ANOVA to assess treatment and exposure time effects, and a Tukey post hoc test to determine significant differences between means. The relationships between epigenetic profiles and physiology were determined using the Pearson correlation coefficients. Significance was set at $P \leq 0.05$.

3. RESULTS

The findings in Tables 1-5 support all the study's objectives. The data presented in the various tables indicate the complexity of the epigenetic and physiological responses of *Arabidopsis thaliana* and *Oryza sativa* to heavy metal stress, particularly cadmium (Cd) and lead (Pb). These facts justify the prospective application of epigenetic markers to create stress-resistant crops and to enhance our analytical understanding of the plant's adaptive mechanisms at the molecular scale.

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Open Access Journal

The experiment involved the interaction of global DNA methylation levels of *Arabidopsis* and *Oryza* plants subjected to heavy metals, as presented in Table 1. The time-dependent methylation levels were found to be time-invariant, with minor differences at the beginning. Nevertheless, the extent to which the DNA methylation decreased over time was extremely high in cadmium-exposed plants. In *A. thaliana*, control plants showed relatively stable methylation levels, with only minor fluctuations around the baseline value of 23.4 ± 1.2% at zero h, increasing slightly to 23.6 ± 1.1% at 24 h (+0.9%) and then decreasing marginally to 23.3 ± 1.2% by 168 h (−0.4%). However, plants exposed to Cd exhibited a pronounced and progressive loss of methylation over time. Starting from 23.5 ± 1.1% at zero h, global 5-mC levels dropped significantly to 20.8 ± 1.4% at 24 h (−11.5%), further declining to 17.6 ± 1.3% at 72 h (−25.1%) and reaching a low of 15.1 ± 1.1% at 168 h, representing a 35.7% reduction compared to baseline.

There was also a reduction in global DNA methylation, mainly caused by lead treatment, but not as serious. *Arabidopsis* showed a 23.6 to 17.6 percent decrease in the degree of methylation and *Oryza*, 26.8 to 22.2 percent, showing that lead causes hypomethylation to a lower degree than cadmium. It was reported by Zhang et al. (2024) that inhibiting DNA demethylation in roots by cadmium stress improves plant tolerance to cadmium toxicity by improving iron nutrition through a feedback mechanism. In summary, exposure to heavy metals, particularly cadmium, results in a significant decrease in global DNA methylation levels in both plant species, which is gradually increased over time.

Table 2 summarizes the changes of histone modifications in *Arabidopsis thaliana* and *Oryza sativa* under cadmium (Cd, 200 mg/kg) and lead (Pb, 500 mg/kg) stresses compared to the control (no stress). The results showed dynamic alterations in some histone marks known to regulate chromatin accessibility and transcriptional activity in response to heavy metal exposure.

The HMA2 gene (metal transporter) at the promoter, which was activated by the histone H3K4me3 mark, was very highly enriched in the *A. thaliana* with Cd treatment. Cd exposure was also observed to significantly elevate the level of H3K4me3 to 1.85 ± 0.20, nearly twice the control baseline of 1.00 ± 0.05. H3K4me3 enrichment also showed less lead induction (1.42 ± 0.18, $p = 0.045$). It implies that HMA2 was transcriptionally induced by Cd and Pb stresses, but Cd induced a larger epigenetic response.

H3K27me3 at PCS1 gene (binding and detoxifying metals) of *O. sativa* was repressed when the plants were stressed as opposed to the control of 1.00 ± 0.04. The H3K27me3 level dropped substantially to 0.58 ± 0.07 ($p = 0.003$) in the presence of Cd stress and to 0.75 ± 0.10 ($p = 0.021$) in the presence of the Pb treatment. The decrease in repressive H3K27me3 indicates that PCS1 was de-repressed in an epigenetic mechanism, thereby promoting phytochelatin formation as well as augmenting metal detoxification, particularly when stressed with Cd.

Another activating mark that was tested on the *A. thaliana* gene (metallothionein gene), which determines the metallothionein (a metal-binding protein) was H3K9ac. The control plants had a baseline of 1.00 ± 0.06 but exposure to Cd led to a high level of induction of the level of H3K9ac to 2.10 ± 0.25 ($p = 0.002$) but Pb exposure induced the level of H3K9ac to 1.60 ± 0.20 ($p = 0.019$). These alterations of acetylation rate suggest a consistent increase in acetylation due to stress of heavy metals and once more Cd was more effective than Pb. Overall, the findings suggest that heavy metals initiate certain histone modification changes of the stress-related genes leading to the expression of transporters (HMA2), metal-detoxifying enzyme (PCS1) and metal-binding protein (MT2A). Cadmium always exhibited much more pronounced epigenetic changes than lead, which is consistent with its greater demethylating capacity in Table 1. This is in agreement with Gallo-Franco et al. (2020) who noted that Cd consistently causes more pronounced epigenetic changes than Pb, aligning with its higher demethylating capacity and greater impact on chromatin structure. These findings support the idea that, as a defense mechanism to cope with a challenge of heavy metal toxicity, plants remodel their chromatin environment by enhancing the expression of defense-related genes.

Table 3 indicates the fold change of various epigenetic regulator genes in response to metal stress, as determined by RT-qPCR, with the base log2 fold change relative to control conditions. In *Arabidopsis thaliana*, exposure to 200 mg/kg cadmium (Cd) resulted in extensive down-regulation of gene DNA methyltransferase MET1 with a mean log2 fold change of -1.8 ($p = 0.002$), indicating reduced expression.

Also suppressed by 200 mg/kg cadmium (Cd) was the CHG methyltransferase CMT3 (-0.9; $p = 0.030$), while the DNA demethylase ROS1 was upregulated (+1.5; $p = 0.010$), suggesting an active demethylation process in response to Cd stress. Lead (Pb) 500 mg/kg significantly did not change the methyltransferase H3K9 SUVH4/KYP (+0.3; $p = 0.28$) but significantly changed the methyltransferase H3K27 JMJ705 (+1.9; $p = 0.001$), which showed that the cadmium stress could induce possible changes in the histone modifications. The histone deacetylase HDA6 in *A. thaliana* and DCL3 in the siRNA biogenesis in rice had no significant difference, and the latter was reduced by 1.2-fold redundancy ($p = 0.015$). These results suggest that stress in metals can influence the expression of epigenetic controllers that regulate gene expression and stress responses in these plants. Chmielowska-Bąk et al. (2023) documented that modulation of epigenetic controllers is a key part of the plant's ability to adapt to and survive in metal-contaminated environments.

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The data in Table 4 show the correlation of the physiological reaction, metal accumulation and epigenetic alteration of *Arabidopsis thaliana* and *Oryza sativa* experiencing metal stress. In *Arabidopsis thaliana*, the shoot biomass was decreased by over 50% when the mean biomass decreased to 0.25 grams per plant compared to the 0.42 grams per plant in cadmium stress and control conditions, respectively. It is worthy to note that this reduction in biomass had a very negative correlation with the extent of global DNA methylation with a Pearson correlation coefficient of 0.78 and a very significant p-value of 0.004, which indicates that the lower or reduced level of methylation is concomitant with the reduced biomass under the condition of extreme stress of heavy metal. Additionally, the amount of cadmium in roots increased to an average of 82.5 µg/g dry weight, and the correlation coefficient is negative ($r = -0.71$, $p = 0.009$), indicating that greater cadmium absorption is associated with lower growth indicators and possibly epigenetic changes.

Table 4: Physiological Responses, Metal Accumulation and Correlation with Epigenetic Marks

Parameter	Species	Treatment (mg/kg)	N	Value Mean ± SD	Pearson r with Global 5-mC	p-value (r)
Shoot biomass (g/g/plant)	*A. thaliana*	Control (0)	8	0.42 ± 0.05	0.12	0.62
Shoot biomass	*A. thaliana*	Cd 200	8	0.25 ± 0.04	0.78	0.004
Root Cd (µg/g DW)	*A. thaliana*	Cd 200	8	82.5 ± 6.8	−0.71	0.009
Leaf chlorophyll (SPAD)	*O. sativa*	Control (0)	8	42.1 ± 2.3	0.09	0.71
Leaf chlorophyll (SPAD)	*O. sativa*	Cd 200	8	28.7 ± 3.0	0.63	0.018
Shoot Pb (µg/g DW)	*O. sativa*	Pb 500	8	45.2 ± 4.9	−0.59	0.026
Electrolyte leakage (%)	*A. thaliana*	Cd 200	8	38.0 ± 5.2	−0.66	0.012

The SPAD values of the chlorophyll content of the leaves of *Oryza sativa* were found to be high in the control plants (mean score of 42.1) and low at 28.7 in the cadmium-treated plants, an indicator of stress-induced chlorosis. The correlation between chlorophyll concentration and global DNA methylation was positive ($r = 0.63$) and statistically significant ($p = 0.018$), indicating that an increase in the levels of methylation is associated with maintenance of the chlorophyll levels under stress. Similarly, at under 500 mg/kg lead exposure, *O. sativa* recorded a negative accumulation of lead in shoot to 45.2 mg/g dry weight, which was negatively correlated ($r = -0.59$, $p = 0.026$), indicating that accumulation of lead in shoots is negatively correlated with health of the plants. Furthermore, the leakage of electrolytes which is a measure of damage to the membrane, rose to 38% and this was negatively correlated with the DNA methylation ($r = -0.66$, $p = 0.012$), which showed that low levels of DNA methylation were associated with the increased cell membrane permeability and cellular damage during the cadmium stress. Similarly, Zabka et al. (2021) reported that Cd-induced hypomethylation correlated with increased oxidative damage, as indicated by higher H2O2 production and DNA damage, both of which compromise membrane integrity and increase permeability in *Vicia faba* root meristem cells.

Overall, the Table 4 data show that changes in global DNA methylation (5-mC concentrations) are significantly related to key physiological parameters, such as biomass, chlorophyll content, metal concentrations, and membrane integrity. These correlations show that epigenetic alterations play a critical role in regulating plant responses to heavy metal stress, influencing tolerance and adaptation, in agreement with Asiminicesei et al. (2024).

The effects of heavy metal treatments, i.e., cadmium (Cd) and lead (Pb), and control on the different plant parameters are summarized in the ANOVA table (Table 5). As can be seen from the analysis, the type of treatment was a significant factor affecting plant responses, with an F-value of 15.34 and a p-value <0.001, indicating that the various treatments had considerable effects on the plant parameters. Also, *variation between Arabidopsis thaliana* and *Oryza sativa* had a considerable influence, with an F-value of 10.45 and a p-value of 0.003. Also, trait and treatment had a significant interaction ($F = 6.18$, $p = 0.009$), indicating that the heavy metal treatment did not have the same effect across the plant traits investigated. The error term is used to explain variability in the data, and the sum of squares of the total represents the overall variation observed. On the whole, these findings indicate that the effects of heavy metal treatments and species variation significantly affect plant responses, and that interactions between these factors contribute to variation in particular traits.

Table 5: ANOVA for Effects of Heavy Metal Treatments on Plant Parameters

Source of Variation	Df	SS	MS	F-value	p-value
Treatments (Control, Cd, Pb)	2	1.245	0.622	15.34	<0.001
Species (A. thaliana, O. sativa)	1	0.684	0.684	10.45	0.003
Trait × Treatment Interaction	2	0.512	0.256	6.18	0.009
Error	42	1.706	0.041	—	—
Total	47	4.147	—	—	—

df = degrees of freedom; SS = sum of squares; MS = mean square; show the pattern of outcomes.

4. DISCUSSION

The research examined the effects of heavy metal stress (cadmium and lead) on two model plants: *Arabidopsis thaliana* and *Oryza sativa*. It examined the functions of histone modifications and the controllers of DNA methylation in plant responses to heavy metal toxicity. The findings indicated that there were strong epigenetic

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alterations associated with heavy metal exposure, affecting growth, photosynthesis, and cellular integrity. The findings indicate the role of epigenetic control in enhancing stress tolerance and maintaining genome stability under heavy metal stress that can be utilized in breeding crops with a higher optimal stress tolerance (Niekerk et al., 2021; Greco et al., 2025; Nugrahini et al., 2025). This process largely focused on histone modifications, which influence the activation or suppression of specific genes under heavy metal stress. An increase in histone marks such as H3K4me3 and H3K9ac at stress-responsive gene loci reproduces an active chromatin state that enhances gene expression, thereby supporting the activity of metal transporters and detoxification mechanisms in response to toxic metal accumulation, in agreement with Wan et al. (2024). Conversely, the repression mark H3K27me3 is linked to elevated phytochelatin production, a key pathway involved in metal detoxification. The changes in these epigenetics are a cascade of coordinated chromatin alterations that control the expression of genes in reaction to metal toxicity. Noor et al. (2024) noted that epigenetic control not only helps mitigate cytotoxic and genotoxic effects but also supports molecular interventions aimed at enhancing crop performance in contaminated environments.

DNA methylation dynamics affect the response of the plant to heavy metals. On cadmium exposure, the activity of DNA methyltransferase was reduced, and the expression of MET1 and CMT3 was down-regulated, in agreement with Greco et al. (2025). Nonetheless, there was an increase in the expression of ROS1, which is a DNA demethylase signifying DNA demethylation activity. This reallocation was probably the cause of stress-induced gene transcriptional stimulation and heightened plant adaptability. JMJ705, a histone H3K27 demethylase, and DCL3, which is associated with methylation by siRNAs, are up- and down-regulated by cadmium stress in *O. sativa*. Such modifications are a sign of repressive epigenetic conditions being reduced to allow transcriptional plasticity. This helps in having phenotypic plasticity and stress memory that enables the plants to be more resistant to environmental challenges (Feng et al., 2016).

Physiological effects of epigenetic changes in the plants were observed on the properties of plants. Cadmium stress in *Arabidopsis* led to decreased shoot biomass, which was positively associated with global DNA methylation levels, implying that a high level of methylation is positive in biomass accumulation under stressful conditions. Root cadmium was negatively correlated with methylation, indicating that metal uptake and transportation increases may be possible at the expense of plant health. Cadmium induced a reduction in the chlorophyll content of the leaf in rice, and the positive levels of methylation indicated that methylation is essential to photosynthesis in the toxic conditions, as indicated by Greco et al. (2025). The experiment found that rice plants under lead stress accumulated large amounts of lead in their shoots, which were negatively correlated with the degree of DNA methylation. This is the sign that lower DNA methylation is correlated with higher lead uptake. These observations highlight the interplay between epigenetic control, metal detoxification, and physiological performance of the plant when subjected to heavy metal stress. Plants use a complex system of epigenetic controls to attenuate gene expression and respond to the toxicity of heavy metals such as lead: histone signaling, DNA methylation, and small RNA signaling. This moderates the growth and detoxification. Biomass and chlorophyll content are positively correlated with DNA methylation, suggesting that DNA methylation could be used as a stress biomarker. Bishop et al. (2024) recorded that rice plants can demethylate methylmercury internally through reactive oxygen species, reducing mercury accumulation in grains and mitigating human exposure risks. Similarly, Popov et al. (2023) cited that studies in various plant species, such as white clover, hemp, kenaf, spinach, and arsenic hyperaccumulators, showed that heavy metal exposure altered global 5-mC levels, which were closely linked to physiological outcomes. For example, decreased 5-mC (hypomethylation) or increased 5-mC (hypermethylation) conferred changes in biomass, chlorophyll content, and membrane stability, as well as metal uptake and distribution. Demethylation, on the other hand, is a method of detoxification but at the expense of plant health, as suggested by Bishop et al. (2024), Iqbal et al. (2024) and Tang et al. (2024). The results could be used to enhance the quality of crops and environmental management.

Epigenetic phenotypes can be applied to breeding programs, including the selection of stress-resistant varieties, including the enrichment of transporter genes by H3K4me3 or the loss of detoxification genes by H3K27me3. It is possible to use epigenome editing technologies to induce specific methylation pattern modifications, making plants more tolerant to heavy metals (Chmielowska-Bąk et al., 2023; Chen et al., 2024; Ibragić et al., 2025). This could be done by epigenetic engineering or chemical priming, which would provide long-term solutions to the problem of soil pollution. One of the sustainable methods of agricultural practices could be epigenetically resilient crops since heavy metals are widespread environmental contaminants that adversely affect crop yields and food security (Charagh et al., 2024; Ghorbani et al., 2024; Mohamed et al., 2025).

The analysis demonstrates that epigenetic reprogramming is very important in the plant's response to heavy metal stress. Histone modifications, DNA methylation, and small RNA processes define physiological traits such as biomass, chlorophyll content, and ion homeostasis (Iqbal et al., 2025; Greco et al., 2025; Lodhi & Srivastava, 2025; Yu et al., 2025). Such similar reactions of plants to heavy metals may appear to resemble each other, but the research demonstrates that species-specific changes in the regulations of epigenetics abide, and crop breeding should be species-specific. The research also offers a foundation to the future research of how these epigenome

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changes can be hereditary and how they can be used in breeding programs. Future studies should aim to determine consistent inherited modifications from one generation to the next and how these interact with genetic variation (Harris et al., 2023; Rahman et al., 2024; Ojeilua et al., 2025). It may result in the development of crops that are resistant to heavy metals over a long period without phytoremediation and chemical applications.

5. CONCLUSION

This paper demonstrates that cadmium and lead exposure lead to extensive epigenetic reprogramming in *Arabidopsis thaliana* and *Oryza sativa, which* can modify DNA methylation, histone remodeling, and gene expression. Such changes are associated with physiological traits such as biomass, chlorophyll content, and ion accumulation, and hence epigenetic control is central to its stress adaptations. The research indicates that plant development under stress conditions relies on the regularity of methylation patterns, whereas detoxification depends on demethylation. The next research directions should involve confirming the heritability of these epigenetic changes and applying them to breed crops resistant to heavy metals, which could help provide long-term solutions to soil pollution and food security issues.

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Ethics Statement: No human participants or animals were involved. The study followed institutional safety protocols.

Author's Contributions: Anthony O. Ukpene conceived and designed the study, coordinated the field experiments, data analysis, and drafting of the manuscript. John C. Morka aided data management, contributed to statistical modeling, and assisted with the review and technical editing of the manuscript. He also helped with the design and analysis of experiments and the interpretation of results, especially regarding yield stability and climate variability. Thelma E. Konyeme was involved in field data collection, soil sample, laboratory analysis, as well as in the materials and methods. All authors have read the manuscript and agreed to submit it to Agrobiology Records.

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