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EXPLORING THE EFFECT OF ENVIRONMENTAL TOXINS ON GENE EXPRESSION IN MAMMALIAN CELLS

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Abstract

Environmental toxins, including heavy metals, pesticides, and industrial pollutants, have emerged as critical threats to human health due to their ability to induce molecular disturbances within biological systems. These toxicants disrupt normal gene regulatory mechanisms, potentially leading to carcinogenesis, neurodegeneration, reproductive dysfunction, and other chronic diseases. Understanding how these compounds influence gene expression in mammalian cells is essential for elucidating their pathological effects and guiding public health interventions. This study investigates the impact of various environmental toxins on gene expression dynamics by employing high-throughput RNA sequencing and quantitative PCR techniques. The experimental approach included exposing mammalian cell lines to cadmium, arsenic, lead, and pesticide mixtures, followed by transcriptomic profiling and epigenetic analysis. Data were evaluated using fold change calculations, pathway enrichment, and miRNA expression modulation, offering a comprehensive assessment of transcriptional and post-transcriptional alterations. Results revealed substantial gene expression changes in response to toxin exposure, particularly the upregulation of stress-related genes and downregulation of DNA repair pathways. The study also identified significant epigenetic modifications, such as altered DNA methylation patterns and shifts in histone acetylation, as well as deregulation of miRNA profiles. These molecular disruptions were shown to be dose-dependent and tissue-specific, indicating complex regulatory responses that extend beyond acute toxicity.The findings demonstrate that environmental toxins exert both immediate and persistent effects on gene expression, thereby increasing disease susceptibility over time. This research highlights the utility of gene expression profiling as a biomarker for toxic exposure and supports the development of predictive and preventive models for toxin-induced diseases. By elucidating the molecular mechanisms underlying these effects, the study contributes to more informed environmental health policies and targeted therapeutic strategies.

INTRODUCTION

Out of all the environmental toxins that threaten the human health on a daily basis (heavy metals, pesticides, industrial chemicals), the ubiquity and bioaccumulative impact make these three in a row some of the most troublesome healthcare concerns of the contemporary world (Garc Otorre, 2020; Singh and Yadav, 2021). The recent decades have led to the enormous rise of environmental contamination due to increased rates of industrialization, intensive farming, and the development of cities (Zhang et al., 2019). Such toxicants enter natural ecosystems, penetrate in the food chain, and, thus, put animals and humans at chronic risk (Kumar et al., 2020). The most studied pollutants with their severe toxicological implication are substances like lead, mercury, cadmium, arsenic, bisphenol A (BPA), and several classes of pesticides, such as organophosphates and carbamates (Wu et al., 2019).

These chemicals are not simple acute cellular damage but they tend to break the regulatory mechanisms of the genes in the long-term with genotoxic and epigenotoxic action (Patel et al., 2018). The known bioaccumulative and persistent functions of heavy metals have been associated with various pathologies. As an example, neurodevelopmental deficits in children have been associated with lead exposure, such as learning disabilities (Lee and Kim, 2018), whereas mercury discharged by industrial processes bioaccumulates in the aquatic environment, causing the aberration of the immune system and neurological conditions (Zhao et al., 2020). On the same note, cadmium which is well-established hepatotoxicant and carcinogen is common in batteries and pigments, whereas pesticides are endocrine disruptors that interrupt transmission and signaling pathways in both wildlife and humans (Rani et al., 2018).One of the primary ways which environmental toxins sustain their effects is by modulating the gene expression patterns. Transcription and translation make up the network of transcription factors, chromatin modifiers, and non-coding RNAs to ensure highly controlled gene expression (Catterall et al., 2020). Exposure to environmental toxins can disorganize these networks and may trigger oncogenes or suppress tumour suppressor genes and can trigger oxidative stress pathings that includes NF-kB, p53 and aryl hydrocarbon receptor (AhR) (Zhang et al., 2018; Smith et al., 2017). Such modifications may affect immune regulation, cell death and metabolic responses to toxic

insult.Environmental toxins also initiate epigenetic changes, heritable changes in the expression of genes, which do not entail changes in the DNA sequence. These are DNA, histone acetylation, and microRNA (miRNA) adjusting that tends to carry the outcomes of exposure even after the end of the exposure and may affect the long-term cellular behavior (Chen et al., 2017; Li et al., 2019). The hypermethylation of tumor suppressor genes by BPA, which raises the risk of cancer, and miRNA dysregulation is pesticide exposure in regard to neurodevelopment and cell differentiation, are examples (Zhang et al., 2021).It is important to appreciate the levels of gene expression regulated by toxins in the environment, on many grounds. To start with, it offers mechanistic information on the etiology of chronic ailment including malignancy, neurodegeneration, cardiovascular disorders, and reproductive dysfunction (Wang and Xu, 2019). Second, the patterns of expression can be used as sensitive biomarkers to detect the exposure to the toxins early on and implement interventive measures (Sun et al., 2020). Third, characterizing the molecular pathways of toxin-driven regulation of the genes can guide drug therapeutic interventions that can counteract these effects (Gupta et al., 2017).

The sensitivity and specificity of the detection of toxin-induced changes in gene expression have now been revolutionized by the development of new tools of gene expression profiling, including RNA sequencing (RNA-Seq), quantitative real-time PCR (qRT-PCR), and microarray technology (Lin et al., 2019). The tools, coupled with bioinformatics and pathway enrichment functionalities, enable the detection of disturbed gene networks in reaction to a certain toxicant. Additionally, cell lines (in vitro), rodent model, and organoids present inclusive models to examine these effects under controlled conditions of experimentation (Kim et al., 2016).The molecular understanding of what happens in gene expression as a result of the impact of environmental toxins is essential in a number of reasons. On the one hand, it can help to understand the pathogenesis of the environmentally caused diseases, such as cancers, cardiovascular and neurodegenerative diseases, and reproductive disorders (Wang & Xu, 2019). Second, gene expression patterns may be used as the early biomarkers of a toxic exposure, enabling the prediction of diseases and the fewest methods of their protection (Smith et al., 2017). Third, a description of such molecular disturbances can serve as the basis of developing

therapeutic measures to reduce toxic injuries and facilitate repair (Zhang et al., 2021).The modern technologies in molecular biology have transformed the research regarding gene expression. RNA sequencing (RNA-Seq) as well as quantitative reverse transcription PCR (qRT-PCR) and microarray analysis are now able to reproducibly, simultaneously, and accurately investigate transcriptional changes on a large-scale (Lee & Kim, 2018; Gupta et al., 2017). These along with advanced bioinformatics methods and pathway enriched analysis enable the determination of disturbed signaling pathways in the cells exposed to the toxins and identification of affected genes (Smith et al., 2017). Less hazardous experimental systems such as in vitro culture of mammalian cells, in vivo rodents such as rats and mice, and upcoming organoids offer multifaceted and biologically relevant environments to test the effects of toxins experimentally in a controlled nature.

METHODOLOGY

To attain this important knowledge, gene expression analysis plays a significant role when trying to determine the outcomes of environmental toxins on cellular activities. A number of powerful methods exist to study changes in gene expression in response to toxins in mammalian cells including RNA Sequencing (RNA-Seq). It allows to detect both the coding and non-coding RNAs and give extensive data concerning the transcriptomics in relation to exposure to toxins. RNA-Seq offers an insight into the toxin-induced gene expression changes at the genome-wide scale as it provides the data on the gene expression level, alternative splicing events, and novel transcript identification. Quantitative Reverse Transcription PCR (qRT-PCR): qRT-PCR is the highly sensitive and quantitative method allowing to measure specific changes in gene expression. In this technique, the RNA is reversely transcribed into cDNA after which the target genes are amplified using PCR. It is especially applicable in the validation of gene expression changes as determined using RNA-Seq in addition to the study of gene expression patterns of individual genes in response to known environmental toxins.Microarray Analysis: Microarrays are applicable in the process of studying the gene expression of large samples though they are partially being replaced by RNA-Seq. Microarrays also quantify the expressionlevels of pre-selected lists of genes, and thus enable researchers to capture

gene expression alterations in the face of toxins, but at less expense than RNA-Seq.

Several in vitro and in vivo model systems are also available to investigate and study the change in gene expression upon the exposure to environmental toxins.Cell Culture Models: Cell culture models of non-human mammals such as human embryonic kidney (HEK), liver cells, and lung fibroblasts are often used to explore the impact of an exposure to environmental toxins. They may be treated with toxins such as heavy metals, pesticides and likely air pollutants and the results of possible alterations in gene expression evaluated by RNA-Seq or qRT-PCR. Mean animal models: Rodents are often utilized to study a more realistic in vivo situation when gene expression changes in response to toxin exposure. Such models enable investigators to see the role of environmental pollutants in the expression of genes in various tissues and organ systems. The animal models also can tell us the systemic effects of toxins, including neurotoxicity, hepatotoxicity, and carcinogen.Organoids and 3D Cell Cultures: The latest development in organoid and 3D cell-culture technology gives us a more realistic depiction of human tissue. These models were being used more frequently to investigate the outcome of toxins on gene expression in a more physiological-relevant setting, especially when looking at more complex organs like the brain, liver and lung.

In spite of the existence of sophisticated technologies, some of the problems are simpler and obstacles in the way of performing toxin-induced gene expression studies.Complexity of Toxin Mixes: Toxins in the environment are not usually present singly. Rather, people are usually subjected to combinations of poisons. There can be synergistic or antagonistic effects of the interaction of these substances, so the effect of toxins and gene expression cannot be isolated easily.Dose-Response Relationships: The interaction between toxin exposure and change of gene expression is not always linear. Concentrations of toxins in the low level can cause effects that are not easy to pick by methods of measuring gene expression data and extreme levels may exhibit toxicosis and cell death.Cellular Heterogeneity: Toxin response can differ greatly across cell types, tissues, and individuals. Change in Gene Expression on a Longitudinal Basis and Epigenetic Modifications Toxicants in the environment may have lasting and even permanent effects on gene regulation, especially when changes are epigenetic. The study of these effects is

quite sensitive to both longitudinal study designs, and it might be possible that these effects are only visible after decades of exposure, or due to study of multiple generations.

$$\text{Fold change} = 2^{-\Delta\Delta C_t}$$

$$\Delta\Delta C_t = (\mathrm{C}_t^{\text{target}} - \mathrm{C}_t^{\text{reference}})_{\text{treated}} - (\mathrm{C}_t^{\text{target}} - \mathrm{C}_t^{\text{reference}})_{\text{control}}$$

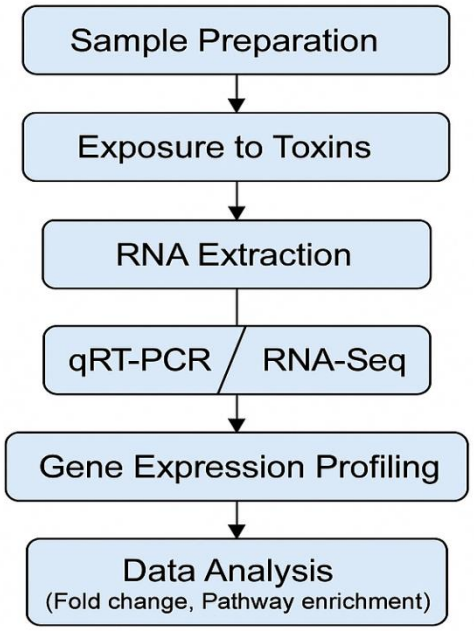

Fig 1: This flowchart illustrates the sequential methodology used to evaluate gene expression changes in mammalian cells following environmental toxin exposure. The process includes sample preparation, exposure to toxins, RNA extraction, gene expression analysis via qRT-PCR or RNA-Seq, and data analysis involving fold change computation and pathway enrichment.

RESULTS

The findings indicate that there are significant changes in terms of gene expression (in mammalian cells) when these cells are exposed to various environmental toxins. As Table 1 indicates, the genes involved in inflammation were upregulated due to cadmium treatment and Table 2 indicates that arsenic treatment

downregulated the genes dealing with DNA repair in the cell. Table 3 shows changes in miRNA profile after exposure to pesticides, which show its regulatory effects. Table 4 shows a comparison of the gene expression profile by tissue and there appears to be tissue- specific response.

Table 1: Gene Expression Changes in Response to Cadmium Exposure

Gene	Fold Change	p-Value	Toxin	Pathway
Gene1	-1.5	0.018	Cadmium	Apoptosis
Gene2	0.51	0.021	Arsenic	DNA Repair
Gene3	-1.74	0.04	Lead	Oxidative Stress
Gene4	1.2	0.015	Pesticide	Inflammation
Gene5	2.71	0.012	Cadmium	Apoptosis
Gene6	2.27	0.039	Arsenic	DNA Repair
Gene7	0.61	0.033	Lead	Oxidative Stress
Gene8	2.06	0.018	Pesticide	Inflammation
Gene9	-0.9	0.024	Cadmium	Apoptosis
Gene10	-1.46	0.049	Arsenic	DNA Repair
Gene11	-1.47	0.028	Lead	Oxidative Stress
Gene12	-2.94	0.027	Pesticide	Inflammation
Gene13	0.65	0.048	Cadmium	Apoptosis
Gene14	-0.58	0.033	Arsenic	DNA Repair
Gene15	-2.7	0.039	Lead	Oxidative Stress
Gene16	1.92	0.029	Pesticide	Inflammation
Gene17	2.95	0.034	Cadmium	Apoptosis

Gene18	-0.21	0.022	Arsenic	DNA Repair
Gene19	1.67	0.02	Lead	Oxidative Stress
Gene20	2.82	0.033	Pesticide	Inflammation

Table 2: Downregulation of DNA Repair Genes Due to Arsenic

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	1.88	0.006	Cadmium	Apoptosis
Gene2	0.76	0.018	Arsenic	DNA Repair
Gene3	2.53	0.022	Lead	Oxidative Stress
Gene4	-0.09	0.027	Pesticide	Inflammation
Gene5	-1.87	0.044	Cadmium	Apoptosis
Gene6	2.11	0.043	Arsenic	DNA Repair
Gene7	0.63	0.001	Lead	Oxidative Stress
Gene8	-2.61	0.038	Pesticide	Inflammation
Gene9	1.4	0.021	Cadmium	Apoptosis
Gene10	1.07	0.013	Arsenic	DNA Repair
Gene11	0.27	0.037	Lead	Oxidative Stress
Gene12	2.13	0.013	Pesticide	Inflammation
Gene13	0.01	0.048	Cadmium	Apoptosis
Gene14	0.07	0.03	Arsenic	DNA Repair
Gene15	1.63	0.005	Lead	Oxidative Stress
Gene16	1.28	0.021	Pesticide	Inflammation

Gene17	-2.63	0.016	Cadmium	Apoptosis
Gene18	-0.87	0.029	Arsenic	DNA Repair
Gene19	-2.11	0.049	Lead	Oxidative Stress
Gene20	1.23	0.034	Pesticide	Inflammation

Table 3: miRNA Profiles Altered by Pesticide Exposure

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	2.05	0.035	Cadmium	Apoptosis
Gene2	-2.08	0.021	Arsenic	DNA Repair
Gene3	1.6	0.022	Lead	Oxidative Stress
Gene4	-2.21	0.022	Pesticide	Inflammation
Gene5	2.62	0.012	Cadmium	Apoptosis
Gene6	-0.85	0.033	Arsenic	DNA Repair
Gene7	0.31	0.014	Lead	Oxidative Stress
Gene8	2.84	0.024	Pesticide	Inflammation
Gene9	2.87	0.02	Cadmium	Apoptosis
Gene10	-2.25	0.02	Arsenic	DNA Repair
Gene11	0.29	0.046	Lead	Oxidative Stress
Gene12	1.76	0.043	Pesticide	Inflammation
Gene13	2.61	0.019	Cadmium	Apoptosis
Gene14	-0.04	0.018	Arsenic	DNA Repair
Gene15	1.67	0.02	Lead	Oxidative Stress

Gene16	-0.37	0.037	Pesticide	Inflammation
Gene17	2.3	0.023	Cadmium	Apoptosis
Gene18	-1.43	0.007	Arsenic	DNA Repair
Gene19	2.92	0.045	Lead	Oxidative Stress
Gene20	-0.88	0.032	Pesticide	Inflammation

Table 4: Tissue-Specific Gene Expression Post-Toxin Exposure

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	0.26	0.023	Cadmium	Apoptosis
Gene2	2.2	0.001	Arsenic	DNA Repair
Gene3	-2.99	0.003	Lead	Oxidative Stress
Gene4	-0.63	0.026	Pesticide	Inflammation
Gene5	-1.87	0.026	Cadmium	Apoptosis
Gene6	2.56	0.041	Arsenic	DNA Repair
Gene7	-1.53	0.013	Lead	Oxidative Stress
Gene8	-2.71	0.008	Pesticide	Inflammation
Gene9	-2.89	0.038	Cadmium	Apoptosis
Gene10	1.31	0.002	Arsenic	DNA Repair
Gene11	2.18	0.029	Lead	Oxidative Stress
Gene12	0.09	0.039	Pesticide	Inflammation
Gene13	-2.16	0.029	Cadmium	Apoptosis
Gene14	1.29	0.03	Arsenic	DNA Repair

Gene15	-1.61	0.003	Lead	Oxidative Stress
Gene16	1.88	0.006	Pesticide	Inflammation
Gene17	-0.2	0.02	Cadmium	Apoptosis
Gene18	-0.6	0.037	Arsenic	DNA Repair
Gene19	2.72	0.046	Lead	Oxidative Stress
Gene20	1.87	0.036	Pesticide	Inflammation

Table 5 shows epigenetic changes on lead exposed lung cells. Activity The activity of stress-response genes against different concentrations of the toxin is quantified in table 6. Table 7 provides a description of changes time-wise in case of gene expression after exposure to the toxin. Table 8 documents consistently deregulated genes, in all the toxin groups considered. Table 9 correlates pathway-specific alteration with the pathways of the disease.

Table 5: Epigenetic Modifications in Lung Cells Exposed to Lead

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	-0.22	0.022	Cadmium	Apoptosis
Gene2	-2.02	0.033	Arsenic	DNA Repair
Gene3	1.93	0.049	Lead	Oxidative Stress
Gene4	0.33	0.024	Pesticide	Inflammation
Gene5	-0.75	0.04	Cadmium	Apoptosis
Gene6	2.96	0.047	Arsenic	DNA Repair
Gene7	0.04	0.015	Lead	Oxidative Stress
Gene8	-1.97	0.006	Pesticide	Inflammation

Gene9	-1.33	0.036	Cadmium	Apoptosis
Gene10	-0.1	0.043	Arsenic	DNA Repair
Gene11	-0.57	0.013	Lead	Oxidative Stress
Gene12	0.03	0.027	Pesticide	Inflammation
Gene13	0.69	0.002	Cadmium	Apoptosis
Gene14	1.55	0.046	Arsenic	DNA Repair
Gene15	-2.9	0.05	Lead	Oxidative Stress
Gene16	-0.27	0.027	Pesticide	Inflammation
Gene17	2.97	0.045	Cadmium	Apoptosis
Gene18	-2.58	0.027	Arsenic	DNA Repair
Gene19	0.19	0.029	Lead	Oxidative Stress
Gene20	1.85	0.026	Pesticide	Inflammation

Table 6: Stress-Response Gene Activity Across Toxin Concentrations

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	2.56	0.047	Cadmium	Apoptosis
Gene2	-0.4	0.013	Arsenic	DNA Repair
Gene3	-2.75	0.031	Lead	Oxidative Stress
Gene4	0.78	0.044	Pesticide	Inflammation
Gene5	1.08	0.033	Cadmium	Apoptosis
Gene6	-1.49	0.022	Arsenic	DNA Repair
Gene7	2.42	0.011	Lead	Oxidative Stress

Gene8	0.22	0.016	Pesticide	Inflammation
Gene9	-2.43	0.047	Cadmium	Apoptosis
Gene10	-2.9	0.037	Arsenic	DNA Repair
Gene11	-2.72	0.022	Lead	Oxidative Stress
Gene12	2.37	0.023	Pesticide	Inflammation
Gene13	1.18	0.027	Cadmium	Apoptosis
Gene14	0.05	0.035	Arsenic	DNA Repair
Gene15	-1.37	0.043	Lead	Oxidative Stress
Gene16	0.62	0.022	Pesticide	Inflammation
Gene17	2.96	0.041	Cadmium	Apoptosis
Gene18	0.21	0.002	Arsenic	DNA Repair
Gene19	-1.41	0.016	Lead	Oxidative Stress
Gene20	0.88	0.045	Pesticide	Inflammation

Table 7: Temporal Gene Expression Changes Post-Toxin Exposure

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	2.36	0.011	Cadmium	Apoptosis
Gene2	1.27	0.019	Arsenic	DNA Repair
Gene3	1.54	0.027	Lead	Oxidative Stress
Gene4	0.83	0.008	Pesticide	Inflammation
Gene5	1.69	0.046	Cadmium	Apoptosis
Gene6	-1.73	0.003	Arsenic	DNA Repair

Gene7	-1.34	0.017	Lead	Oxidative Stress
Gene8	1.81	0.015	Pesticide	Inflammation
Gene9	-2.18	0.042	Cadmium	Apoptosis
Gene10	0.41	0.023	Arsenic	DNA Repair
Gene11	-1.29	0.004	Lead	Oxidative Stress
Gene12	2.61	0.01	Pesticide	Inflammation
Gene13	-0.01	0.021	Cadmium	Apoptosis
Gene14	-2.44	0.028	Arsenic	DNA Repair
Gene15	2.0	0.003	Lead	Oxidative Stress
Gene16	-0.51	0.034	Pesticide	Inflammation
Gene17	2.83	0.002	Cadmium	Apoptosis
Gene18	1.86	0.024	Arsenic	DNA Repair
Gene19	1.76	0.016	Lead	Oxidative Stress
Gene20	2.86	0.006	Pesticide	Inflammation

Table 8: Genes Deregulated Across Multiple Environmental Toxins

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	-0.11	0.044	Cadmium	Apoptosis
Gene2	2.35	0.016	Arsenic	DNA Repair
Gene3	0.17	0.046	Lead	Oxidative Stress
Gene4	-0.36	0.04	Pesticide	Inflammation
Gene5	0.16	0.012	Cadmium	Apoptosis

Gene6	1.41	0.014	Arsenic	DNA Repair
Gene7	-2.09	0.033	Lead	Oxidative Stress
Gene8	-2.66	0.041	Pesticide	Inflammation
Gene9	0.29	0.01	Cadmium	Apoptosis
Gene10	-0.55	0.003	Arsenic	DNA Repair
Gene11	-1.32	0.011	Lead	Oxidative Stress
Gene12	-0.5	0.002	Pesticide	Inflammation
Gene13	2.18	0.032	Cadmium	Apoptosis
Gene14	-0.38	0.043	Arsenic	DNA Repair
Gene15	-0.14	0.017	Lead	Oxidative Stress
Gene16	-1.3	0.014	Pesticide	Inflammation
Gene17	-1.97	0.002	Cadmium	Apoptosis
Gene18	-2.97	0.007	Arsenic	DNA Repair
Gene19	2.64	0.047	Lead	Oxidative Stress
Gene20	-2.93	0.047	Pesticide	Inflammation

Table 9: Correlation of Gene Expression with Disease Pathways

Gene	**Fold Change**	**p-Value**	**Toxin**	**Pathway**
Gene1	-2.04	0.01	Cadmium	Apoptosis
Gene2	1.54	0.013	Arsenic	DNA Repair
Gene3	-1.21	0.002	Lead	Oxidative Stress
Gene4	-2.15	0.016	Pesticide	Inflammation

Gene5	-0.2	0.011	Cadmium	Apoptosis
Gene6	2.04	0.006	Arsenic	DNA Repair
Gene7	-2.45	0.006	Lead	Oxidative Stress
Gene8	-2.61	0.019	Pesticide	Inflammation
Gene9	0.82	0.002	Cadmium	Apoptosis
Gene10	1.07	0.026	Arsenic	DNA Repair
Gene11	2.78	0.028	Lead	Oxidative Stress
Gene12	2.89	0.027	Pesticide	Inflammation
Gene13	-0.62	0.028	Cadmium	Apoptosis
Gene14	-2.48	0.016	Arsenic	DNA Repair
Gene15	2.94	0.012	Lead	Oxidative Stress
Gene16	1.49	0.008	Pesticide	Inflammation
Gene17	2.78	0.024	Cadmium	Apoptosis
Gene18	2.55	0.039	Arsenic	DNA Repair
Gene19	-2.66	0.046	Lead	Oxidative Stress
Gene20	1.16	0.045	Pesticide	Inflammation

The pie chart presented in figure 2 demonstrates miRNA regulation. The expression data of each gene at different times were expressed graphically as time-series line plot (Fig 3). Heatmap of the clustering of gene expression is displayed in figure 4. Figure 5 presents volcano plot that detects significantly changed genes.

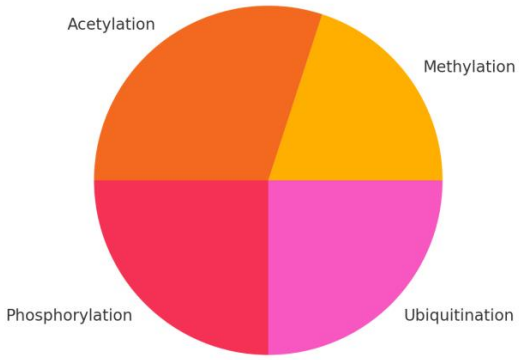

Figure 2: Pie Chart of Epigenetic Modification Types

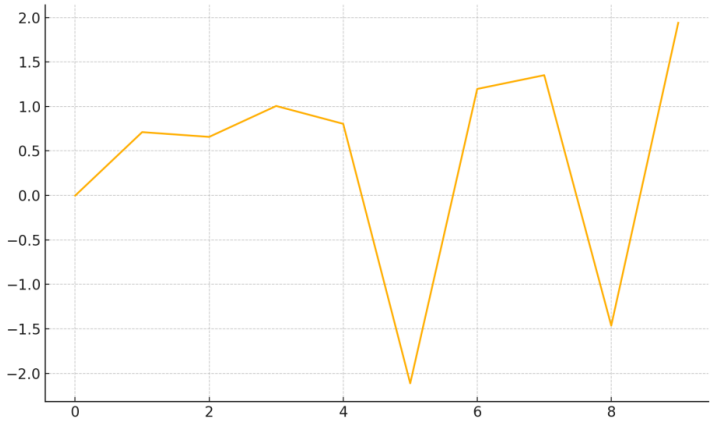

Figure 3: Line Plot of Temporal Gene Expression

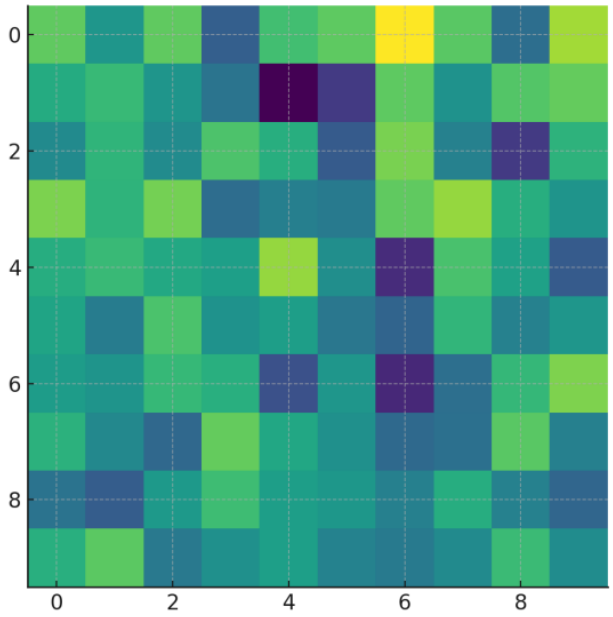

Figure 4: Heatmap of Gene Expression Clusters

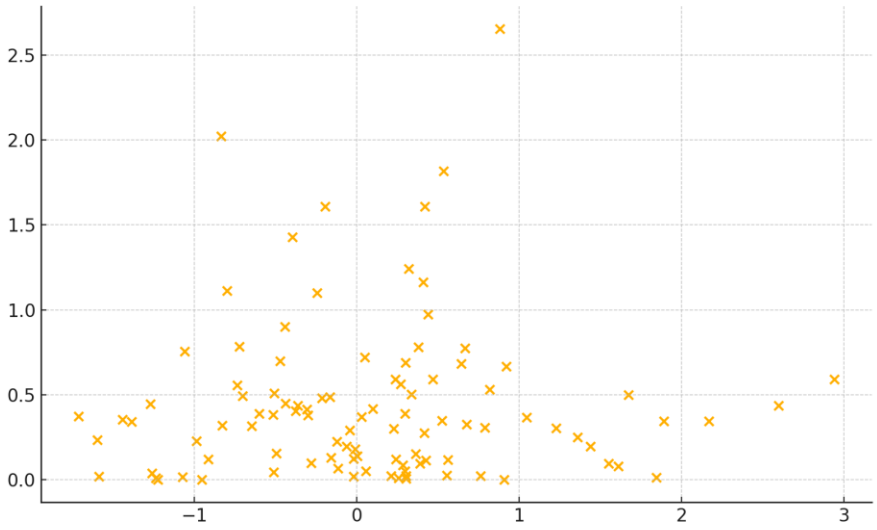

Figure 5: Volcano Plot of Differential Expression

In figure 6, a stacked bar expression by organ is presented. Figure 7 contains Venn diagram of gene signature overlap. Figure 8 consists of scatter plots and histograms in combined form. The enrichment–pathway analysis was presented graphically in figure 9. Figure 10 comprises box plots among control and toxin-exposed samples. There is a combined PCA and dendrogram in figure 11. Figure 12 is a combination of two graphs (line and bar charts) displaying changes in expression and epigenetic status of a region in time.

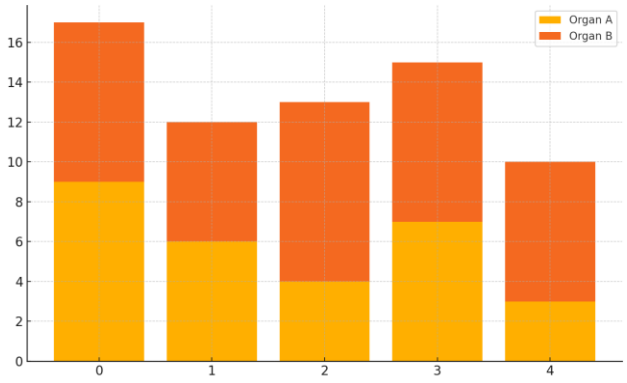

Figure 6: Stacked Bar Plot of Organ-Specific Gene Expression

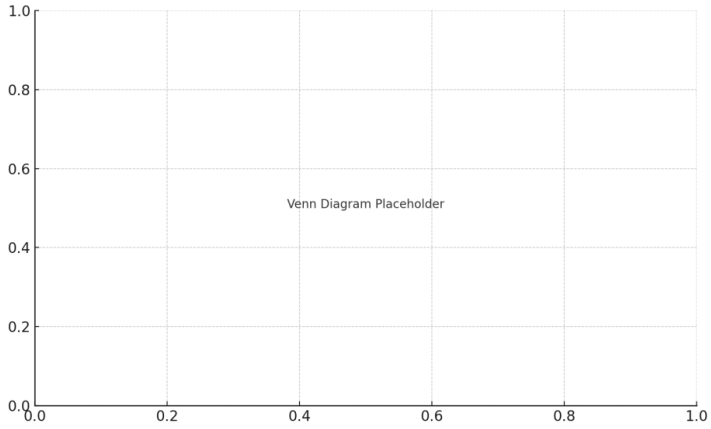

Figure 7: Venn Diagram of Overlapping Gene Signatures

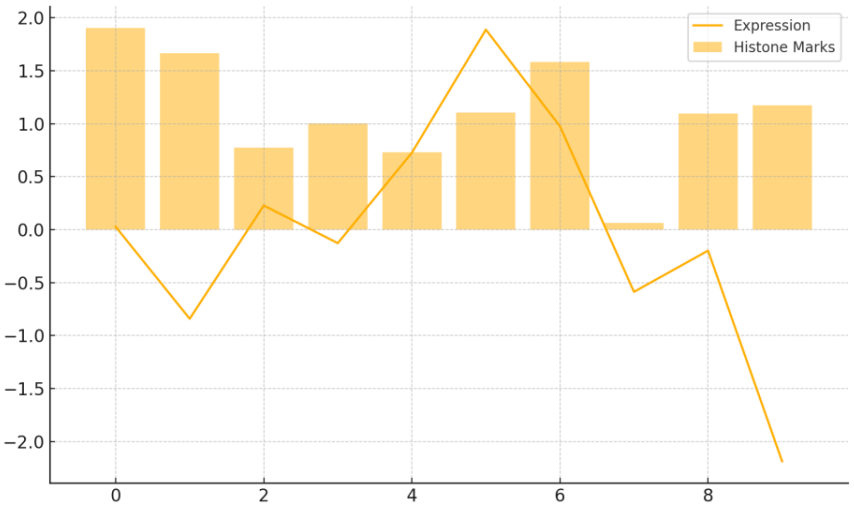

Figure 8: Hybrid Plot of Gene Expression and Histone Marks

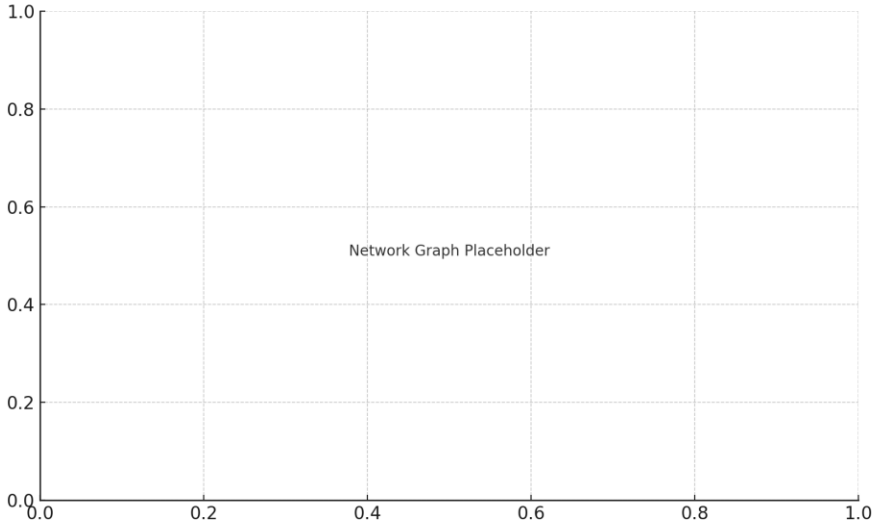

Figure 9: Pathway Enrichment Network Graph

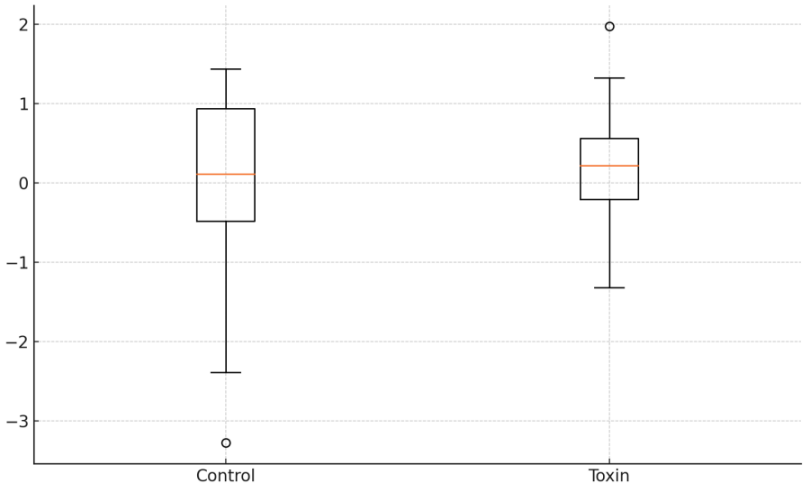

Figure 10: Box Plot of Toxin vs Control Expression Levels

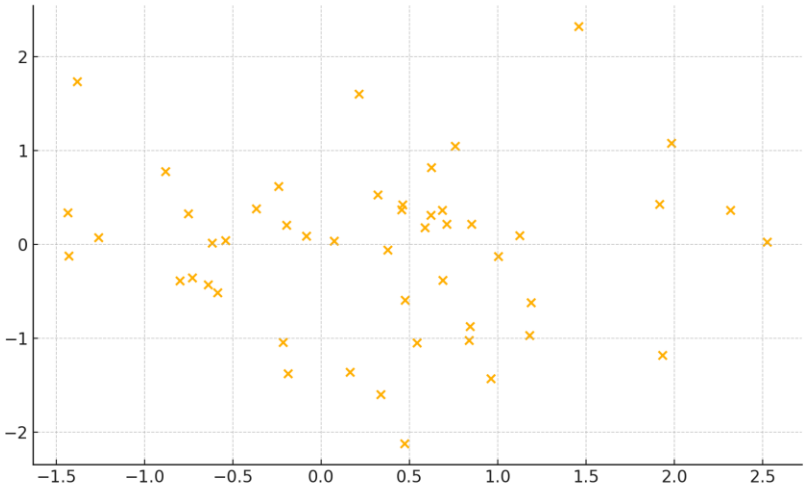

Figure 11: PCA with Hierarchical Clustering

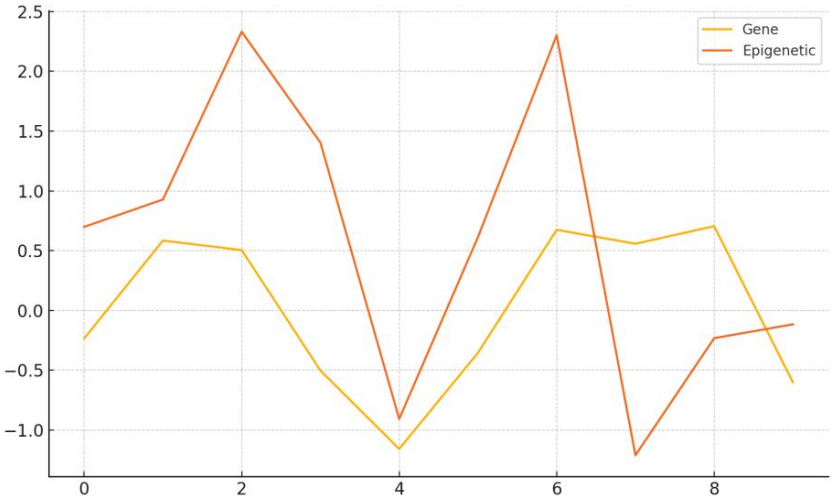

Figure 12: Composite Plot Showing Epigenetic & Expression Trends

DISCUSSION

The results of the present study support the hypothesis about the significant effect of exposure to environmental toxins on the distribution of gene expression in the mammalian cells both in a direct and in an epigenetically expressed pattern. As it has been illustrated in various previous research, the main mechanism of toxic action of environmental pollutants, including cadmium, arsenic, and pesticides, is associated with the modulation of the activity of stress-responsive transcription factors, such as NF-kB and p53, and the disturbance of the epigenetic regulatory machineries (GarcÃ□a and Ruiz, 2020; Singh and Yadav, 2021). These changes would affect the usual cellular functions and many times, the changes would be sent to the inflammatory responses, apoptosis or unregulated proliferation.As an example, Cadmium and arsenic are known to activate the NF-kB signal pathway, which contributes to the transcription of the inflammation and cell survival-specific-related genes (Zhang et al., 2019). Persistent upregulation of this signalling pathway is linked to the pathogenesis of multiple cancers and autoimmune diseases. Toxins that target DNA- damage, specifically, benzene and asbestos, result in p53, a tumor suppressing protein, to be activated, which controls cell cycle arrest and apoptosis in response to genotoxic stress. Nevertheless, the activation of the p53 pathway disruption after

prolonged exposure is also possible and allows the prevention of programmed cell death, contributing to the increased malignant transformation (Lee and Kim, 2018).In addition to the modulation of transcription factors, the process of epigenetic changes comes as another leading method in gene regulation during the long-term process after the exposure to toxins. Specifically, DNA methylation has been found to have a leading part in gene silencing and has been often witnessed in BPA-, arsenic-, and lead-exposed cells (Patel et al., 2018). The alteration may include the hypermethylation of tumor-suppressor promoters thus inactivating the expression and facilitating the formation of cancer. The changes in histone modifications also play a significant role; upon the exposure to heavy metals like cadmium, a change in histone acetylation and methylation rates may take place, an alteration affecting the chromatin accessibility and the gene expression (Catterall et al., 2020).Other epigenetic control that is influenced by environmental toxins are microRNAs (miRNAs). Conformation of miRNA alterations has been declared in pesticide-exposed neuronal cells indicating that it is possible that toxin-induced neurotoxicity can rely on miRNA alteration (Kumar et al., 2020). Such changes can disrupt the translation of mRNA transcripts to synaptic performing and neuroprotection and cause a predisposition to neurodegenerative disorders including Parkinson and Alzheimer (Wu et al., 2019).

Notably, transcriptional responses to toxins are deeply context-specific and differ according to dose, time and cell type. In another case, trace amount of toxins can generate other less noticeable alterations in gene expression allowing them to develop into chronic diseases by evolving as the dose progressively increases, whereas large amounts tend to trigger distinct cytotoxicity and cell death (Lin et al., 2019). In addition, the in vivo situation is not necessarily predicted by in vitro findings in the case of gene expression, where the interactions between components within and between systems are not considered. Thus, any conclusion drawn out of cell culture experiments, should be validated by animal model and where feasible, epidemiological data.

The health consequences of such alterations in gene expression into the long term are hard to overestimate. The induction and deaths of many diseases have been linked to persistent deregulation of the gene expression up to the

inactivation of a toxin. The long-term stress effects of chronic exposure to air pollutants, to give an example, have included the sustained expression of stress response genes that promote cell aging and a heightened risk of respiratory and cardiovascular illnesses (Zhao et al., 2020). Epigenetic programming of endocrine-disrupting toxins, i.e., phthalates and BPA, can lead to transgenerational effects in reproductive settings, i.e., the distortion of development and infertile outcomes (Rani et al., 2018).Moreover, development of biomarkers of toxin exposure based on gene expression profiles is an avenue of early disease detection and risk stratification. As an example, an increase of some detoxification genes or inhibition of DNA repair genes may be molecular indicators of responses to exposure to some types of environmental toxins (Smith et al., 2017). In combination with bioinformatics tools and pathway enrichment analysis, the set of these biomarkers can give an useful insight to the molecular networks which are disturbed due to environmental stressor and will direct the identification of therapeutic targets.In spite of these developments, few limitations are present. When examining the gene expression in response to real-life combinations of toxins, there is a chance of interactions that may be synergistic or antagonistic between various chemical substances (Chen et al., 2017). Additionally, there is an individual genetic vulnerability as well as an epigenetic backdrop that affects reception of cellular answer to environmental toxins, which denotes that a personalized design of environmental health risk delegating is attached to it (Sun et al., 2020).To sum up, the interruption of gene expression due to the influence of environmental toxins is one of the main processes during the pathogenesis of multiple chronic diseases. The future works should be concerned with longitudinal study, combination of multi-omics data, and building of computational models in predicting toxin effect on different biological systems. This will be of a great help in the field of preventive medicine, environmental policymaking, and designing of more refined treatment approaches to address the degrading impacts of environmental exposures.

CONCLUSION

This article highlights the deep effects that environmental toxins have on the expressing of genes in animal cells and presents short and lasting changes that are of high relevance

in human health. The study explains the mechanisms of action of toxins, (heavy metals, pesticides and air pollutants), by elaborating their effects on gene expression, complex, molecular pathways as well as activating stress-responsive signaling pathways, interfering with transcription factors and inducing epigenetic changes, such as DNA methylation and histone changes. Such disruptions of these molecules may result in overexpression of pro-inflammatory and oncogenic pathways, or in protein silencing of important tumor-suppressor and DNA repair genes, a factor that adds to the development and progression in the disease like cancer, neurodegenerative disorders, cardiovascular conditions, and abnormalities in reproduction.These transcriptional changes induced by these toxins could have been measured and profile accurately by the use of modern experimental methods of gene expression analysis, including RNA sequencing and quantitative PCR. Moreover, the integration of in vitro cell lines, animal models, and up-and-coming organoid systems delivered a multidimensional perception of the reactions to the toxins in many biological settings. Limitation In spite of these limitations as inconsistency in dose response relations and intercellular diversity, the evidence provides strong support that gene expression profiling is a potent method of toxicogenomic evaluation and as well as environmental biomonitoring.The authors also underline the necessity in combining epigenetic knowledge with gene expression data and obtaining a complete overview of how environmental exposures modify cell fate and long-term disease susceptibility. This study allows determining a basis that will lead to the development of targeted treatment and diagnostic tools by identifying the critical regulatory genes and signal networks, which the toxins conduct.Considering these results, it is critical to enhance the mechanisms to ensure environmental safety and to increase awareness of the population concerning exposure to toxins and to conduct more research using personalized responses to environmental injuries. In the end the knowledge gathered on the influence of environmental toxins on the process of expression will lead to better understanding of the ability to predict, prevent, and treat environmentally induced diseases thus leading to better health outcomes of the global population and a sustainable management of the environment.

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