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Amino Acid Profile of MCF- 10A Cells Following Agrochemical Exposure: Metabolomics ApproachEkwempu, A.I.^{1*}, Oluboyo, A.O.², Odewusi, O.², Lorenzi, PL.³

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Author for Correspondence*: adaekwem@yahoo.com. <https://dx.doi.org/10.4314/sokjmls.v10i1.3>**Abstract**

Agrochemicals are commonly used by farmers for agricultural purposes such as weed control, pest control, and improvement in farm produce. Globally, there are significant health problems associated with the inappropriate handling of agrochemicals. Metabolomics has undergone over 20 years of continuous innovation, it has created the most advanced system for measuring and understanding metabolites which are the end products of environmental and biological interactions. They also serve as biomarkers. Studies from the past decades have proven the important role of amino acids in cancer metabolism in both a tumorigenic and tumor-suppressive way. This experimental study was carried out at Metabolomics unit of The University of Texas, MD Anderson Cancer Center Laboratory, USA. We treated the non-malignant breast cell line MCF10A with three (3) commonly used agrochemicals (Atrazine, Chlorpyrifos, and Paraquat dichloride) in Nigeria. Concentrations that are permitted in drinking water was used during the exposure. The resulting changes in the amino acid were analyzed and reported. Our initial focus was on amino acid measured by ion chromatography-mass spectrometry (IC-MS) using established methodology. The metabolomic patterns indicated that amino acids like methionine sulfonate, cysteine, kynurenine, acetylcholine were depleted by all agrochemicals while proline was increased. On the other hand, Chlorpyrifos and paraquat dichloride showed overall similar impact on cell metabolism on day 1, and on day 2 of exposure, but atrazine was different on day 2. Chlorpyrifos day 2 and atrazine day 1 showed the presence of different amino acids there seems to be consensus on “day 2” effect. In this study, amino acids: methionine sulfonate, cysteine, kynurenine, acetylcholine was depleted by all agrochemicals. The finding of an increase in the proline amino acid for both chlorpyrifos and paraquat

dichloride suggests that these chemicals exert similar actions on cells. This was however different for glutamine amino acid which was increased during atrazine exposure, but decreased during chlorpyrifos and Paraquat exposure. This study therefore suggests that the metabolomic pattern for chlorpyrifos and Paraquat exposure are similar.

Keywords: *Agrochemicals, metabolomics, MCF10A cell line, Amino acids*

Introduction

Agrochemicals are commonly used by farmers for agricultural purposes such as weed control, pest control, and improvement in farm produce. Sub-Saharan Africa was reported to have the highest number of deaths precisely in Western Africa (20.1 per 100,000/year) which was attributable to environment. This reflects both infectious diseases, non-communicable diseases and injuries. Africa in recent years has recorded several industrial and agricultural developments associated with heavy metals, agrochemical use including pesticides, air pollution, water contaminants, and waste generation which represents hazardous exposures (Impouma, 2020). Different researchers (Fitzmaurice *et al.*, 2018) have reasoned that these chemicals may also have effect on humans since they eliminate other living things. Farmers who have little or no knowledge mechanism of action for these chemicals are responsible for their utilization on agricultural farms. The use of these chemicals has been linked to diverse health and environmental challenges which is as a result of direct physical contact during application. In addition, agrochemicals are known to drift from fields where they have been applied thereby contaminating foods and drinking water thereby increasing the risk for health related problems (Ekwempu, 2019). Globally, there are significant health problems associated with the inappropriate handling of agrochemicals. Reports

from WHO, 2017 indicates that some health issues implicated with the use of agrochemicals include: different types of cancers (Breast, lungs, gastrointestinal tract, etc.), male/female infertility disorders, and chronic noncommunicable life-threatening public health issues such as organ/endocrine disorders (Wimalawansa & Wimalawansa, 2014).

The field of metabolomics is a relatively new field of biological science coined from the metabolome in 1998 and it focuses on analysis of metabolites (Subramani *et al.*, 2022). It describes the total number of metabolites present within the cell, tissue, organ, or organism which have a wide range of functions also known as metabolome. The message in the DNA (genome) of an organism are transcribed into RNA (transcriptome), translated into protein (proteome) and finally results in the formation of small molecules (result of metabolism) known as metabolites (Yang *et al.*, 2020; Wan *et al.*, 2021) (metabolome). This implies that any change in the gene, be it mutation, over-expression, or under-expression, affects the metabolomics profile of an organism. A vast majority of diseases, including cancer, are the result of alteration in gene expression profile, e.g., BRAC1/BRAC2 genes are the most mutated genes in hereditary breast cancer (Ngrid *et al.*, 2001). Researchers have reported that alterations in genes could cause changes in the metabolic profile, and these changes eventually could facilitate cancer development (Nittoli *et al.*, 2018).

Various studies have focused on the metabolic profile of several biological samples, including lipidomics (lipids), labelled substrates (e.g., ¹³C labelled glucose), volatome (volatile organic metabolites), and metabolites resulting from Krebs cycle (Cala *et al.*, 2019; Chen *et al.*, 2019). Identifying metabolites early in disease onset, metabolic reprogramming, cancer typing, staging and therapeutic intervention response has been shown to reduce mortality associated with breast cancer (Chen *et al.*, 2019). There is growing evidence that mapping out the pathway for Krebs/TCA cycle, helps in understanding the energy production and macromolecule synthesis that occurs in cancer cells. This is particularly true with dysregulated oncogene and tumour suppressor expression (Willmann *et al.*, 2016). There has been a rapidly growing number of metabolomic studies over the past few years all aimed at discovering new biomarkers for breast cancer diagnosis using different biological matrices.

It has been confirmed that breast cancer is associated with alterations in metabolism, but a current knowledge gap is whether agrochemical exposure induces metabolic changes that promote breast cancer. To address that gap, here we tested the hypothesis that selected pesticides modulate amino acid metabolism.

Materials And Method

The research study design was experimental using commercially prepared cell line, MCF10A, a human breast epithelial cell line which is the most commonly used normal breast cell model. This study design was set up to help establish a better understanding of the Amino acid profile of MCF 10A cell lines exposed to selected agrochemicals. Breast Cells were exposed to three (3) commonly used agrochemicals (Atrazine, Chlorpyrifos, and Paraquat dichloride) in Nigeria (Udoh & Gibbs, 2022). This study was carried out at The University of Texas, MD Anderson Cancer Center Laboratory, Metabolomics unit in United States of America. Amino acids were separated and detected using ion chromatography-mass spectrometry (IC-MS) using established methodology. The manufacturer's instructions were followed (Horvath *et al.*, 2017). Methods of choice were ion chromatography-mass spectrometry (IC-MS) as described in PMID: 39333384. Statistical analysis was carried out using Metabo Analyst 6.0 a web-based platform dedicated for comprehensive metabolomics data analysis.

Results and Discussion

Metabolites associated with the different agrochemicals and time point of exposure were determined using the relative clustering abundance based on Hierarchical clustering heatmaps which showed different metabolites at different time points. The Next-generation heatmap (NGCHM) software showed plots showed covariate bars, and link-outs that enabled deep exploration of patterns and associations in heat maps. The metabolomic patterns indicate that amino acids like methionine sulfonate, cysteine, kynurenine, acetylcholine were depleted by all agrochemicals. On the other hand, Chlorpyrifos and paraquat dichloride showed overall similar impact on cell metabolism on day 1, and on day 2, but atrazine was different on day 2. Chlorpyrifos day 2 and atrazine day 1 showed the presence of different amino acids there seems to be consensus on "day 2" effect, this is shown in figure 1. In this study, amino acids: methionine sulfonate, cysteine, kynurenine, acetylcholine was depleted by all agrochemicals (Figures 2,3,4,5). The amino acid metabolism pattern in our study indicates that Choline, Carnitine and Proline were increased during Chlorpyrifos exposure (Figure 6). On the other hand, exposure to Chlorpyrifos and paraquat dichloride exposure showed overall similar metabolomic pattern for amino acids during different days of exposure (figure 7), suggesting that cell metabolism was similar, however exposure with atrazine was different on the second day of exposure. Creatinine, glutamic acid and Glutamine were predominantly detected for Atrazine exposure while Carnitine, proline were predominant for paraquat exposure. Branched chain

amino acids eg Valine, leucine, and isoleucine, are crucial amino acids with significant implications in tumorigenesis across various human malignancies. Increased levels have been associated with tumour growth inhibition; this agrees with our work which showed an association with biosynthesis. Findings of an association between degradation of valine, leucine and iso leucine agrees with other studies which reported a deficiency .

These variations in the predominant amino acids detected maybe associated with the fact that the chemicals have different mode of action. In a metabolomic study carried out in China to determine the effect of Chlopyrifos on rice plant metabolism. The researchers reported that chlorpyrifos could affect the metabolic profiling of rice tissues. Some metabolites with significant changes found in their study, were mainly organic acids, amino acids, lipids, polyphenols, and flavonoids. Their work also showed that when compared to the control, the content of glutamate family amino acids were significantly disturbed by chlorpyrifos, while defense-related proline and glutathione were significantly increased; however, glutamic acid, N-acetyl-glutamic acid and N-methyl-glutamic acid were significantly decreased. The finding of an increase in the proline amino acid for both

chlorpyrifos and paraquat dichloride suggests that these chemicals exert similar actions on cells. This was however different for glutamine amino acid which was increased during atrazine exposure, but decreased during chlorpyrifos and Paraquat exposure. This study therefore suggests that the metabolomic pattern for chlorpyrifos and Paraquat exposure are similar.

Over the years and with the introduction of tumor biology and tumor therapy, amino acid metabolism have been recognized as an important team player in tumorigenesis and tumor immunity (Chen *et al.*, 2024). Zhang *et al.* (2022) reported carnitine levels were associated with breast cancer development in addition, recent research has shown that choline metabolism undergoes significant changes in cancer, resulting in a heightened demand for choline and its metabolites (Van *et al.* , 2023). Their mode of action relates to their ability to act as sources of nutrients and signaling molecules, for the regulation of gene transcription and epigenetic modification. Understanding amino acid metabolism during exposure to agrochemicals can provide new ideas for tumor treatment and become an important therapeutic approach for breast cancer management (Nolasco *et al.*, 2023).

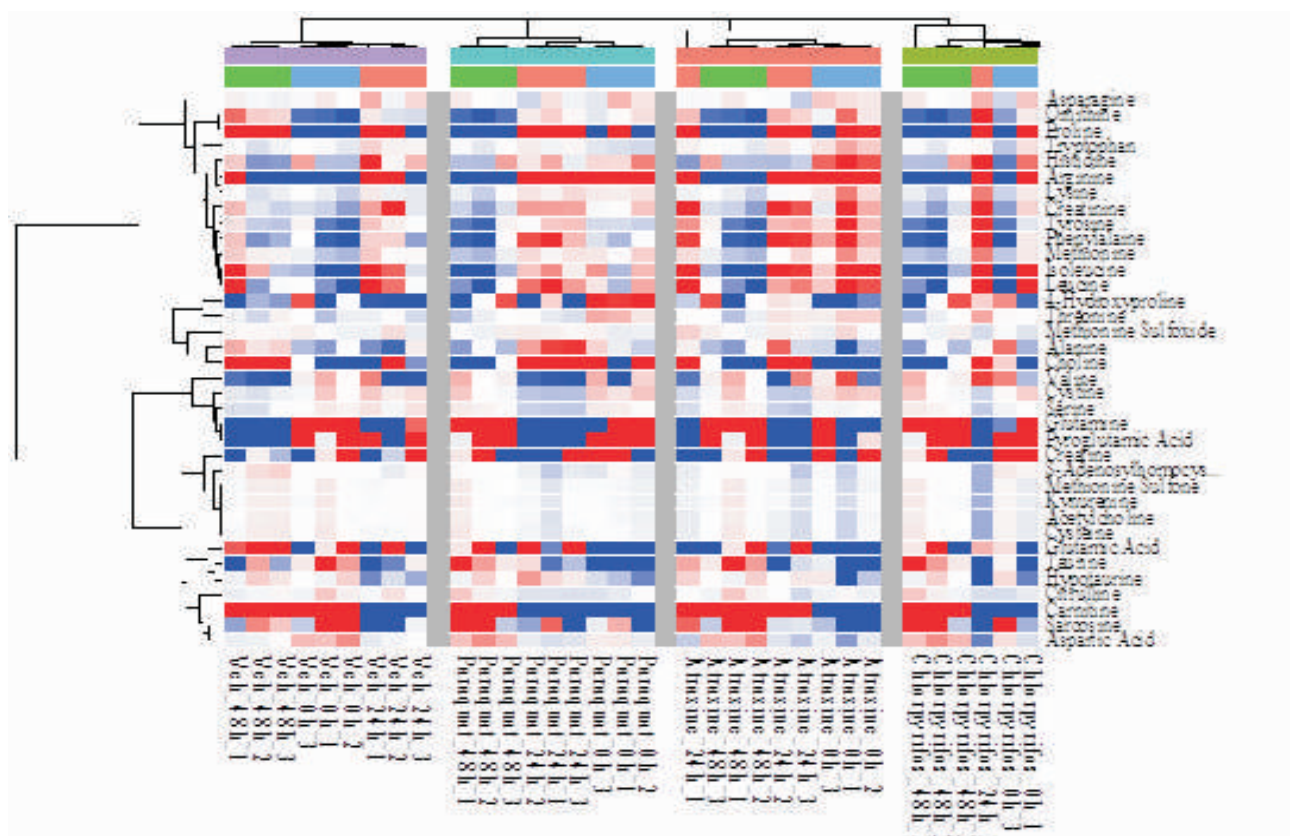


Figure 1: Heatmap showing amino acids and agrochemical exposure

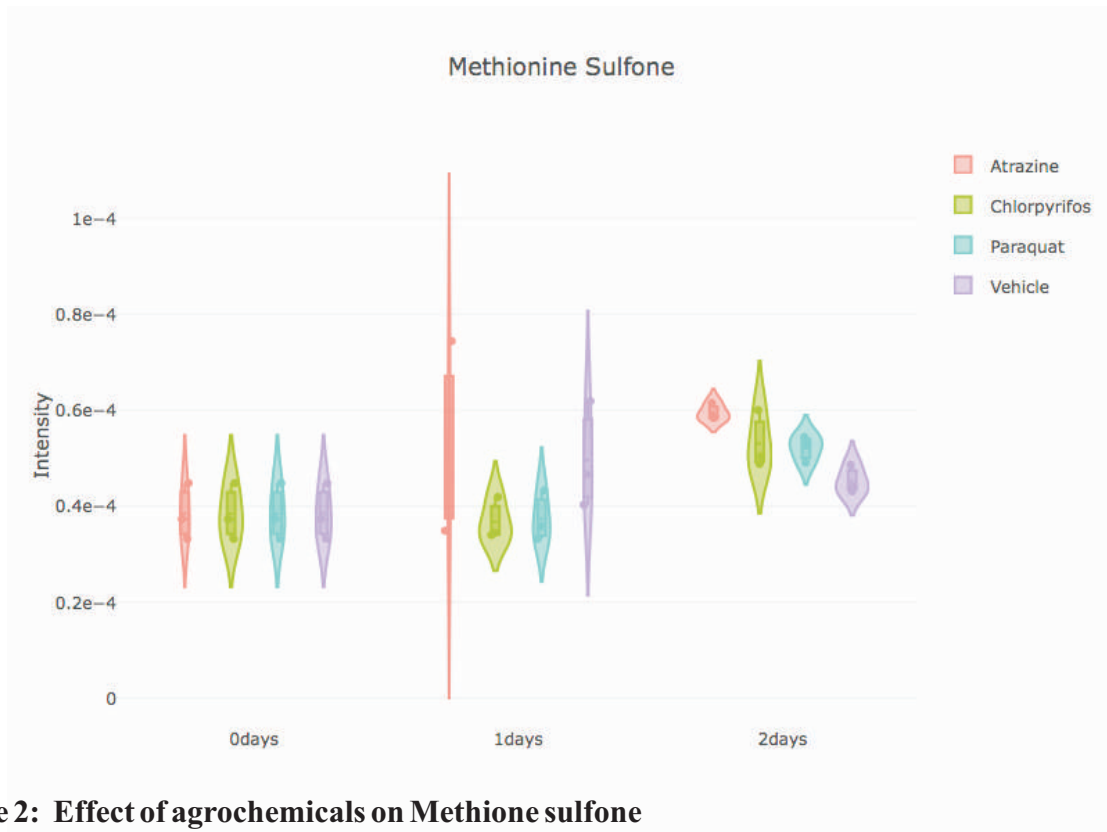


Figure 2: Effect of agrochemicals on Methionine sulfone

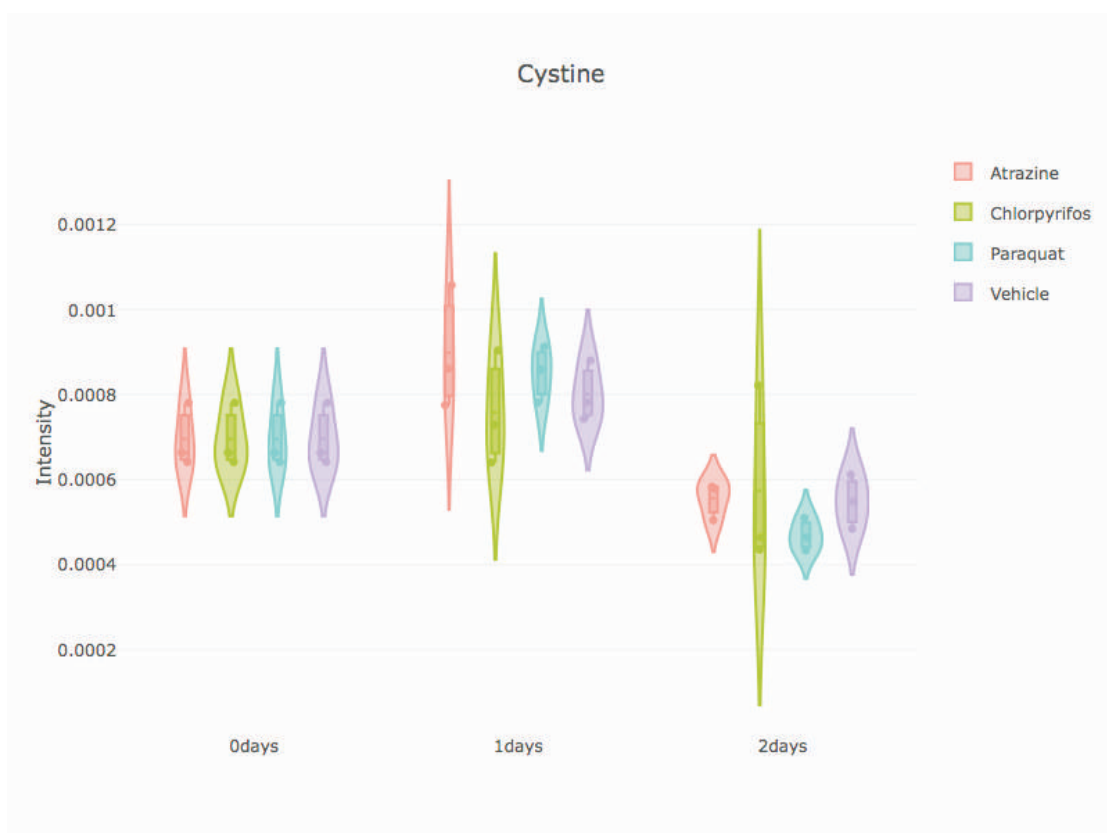


Figure 3: Effect of agrochemicals on Cystine

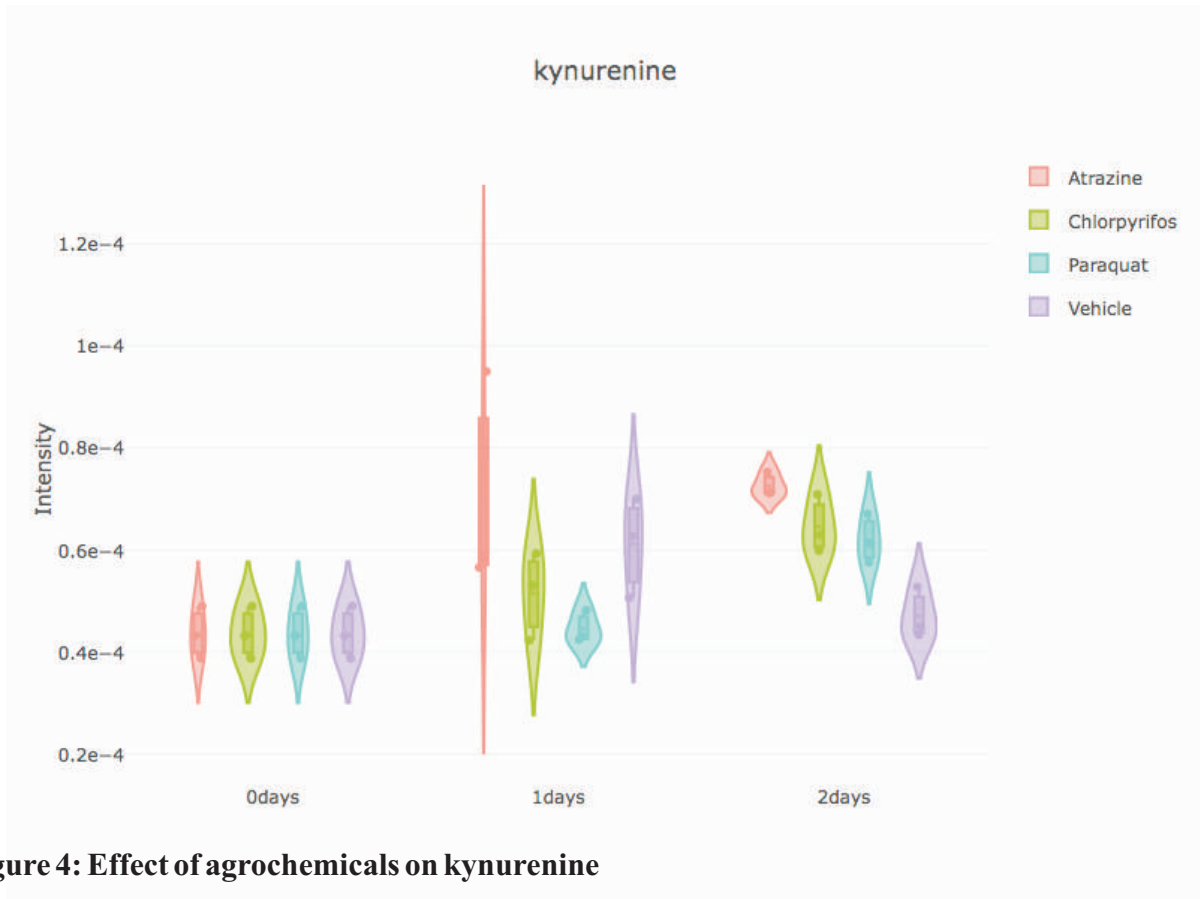


Figure 4: Effect of agrochemicals on kynurenine

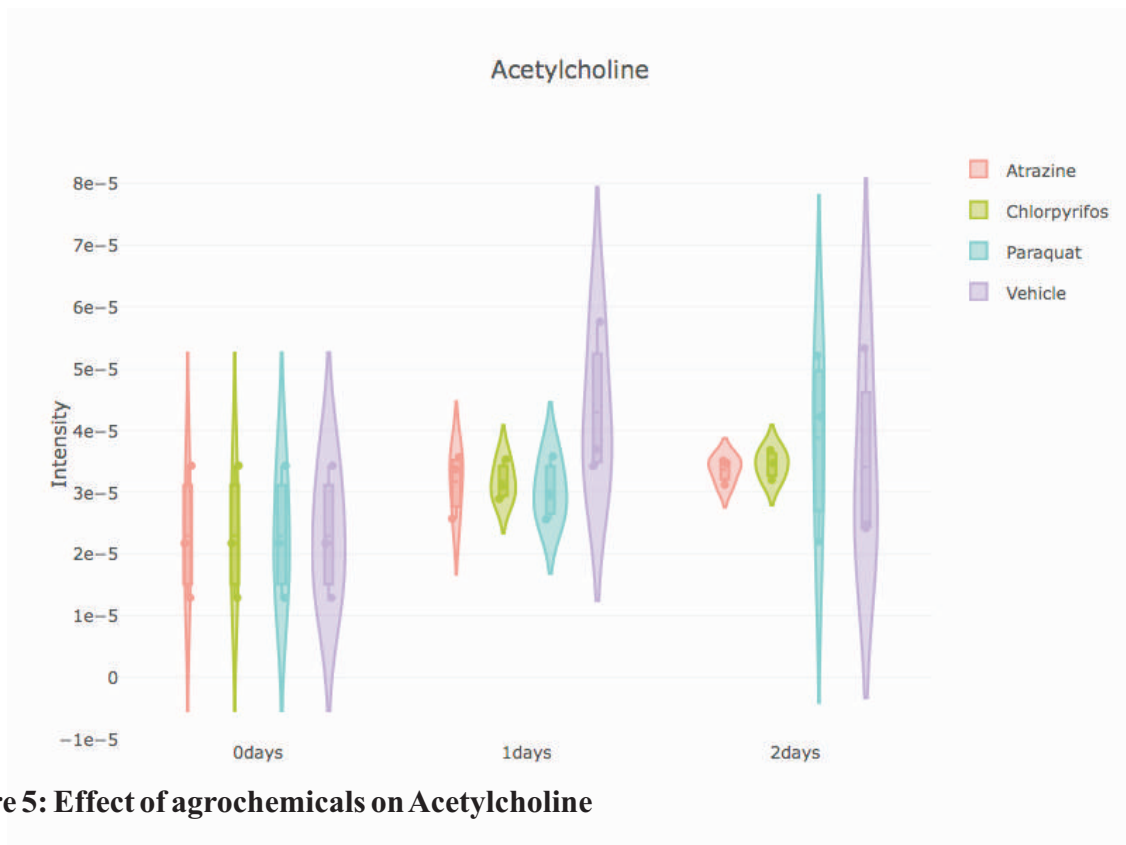
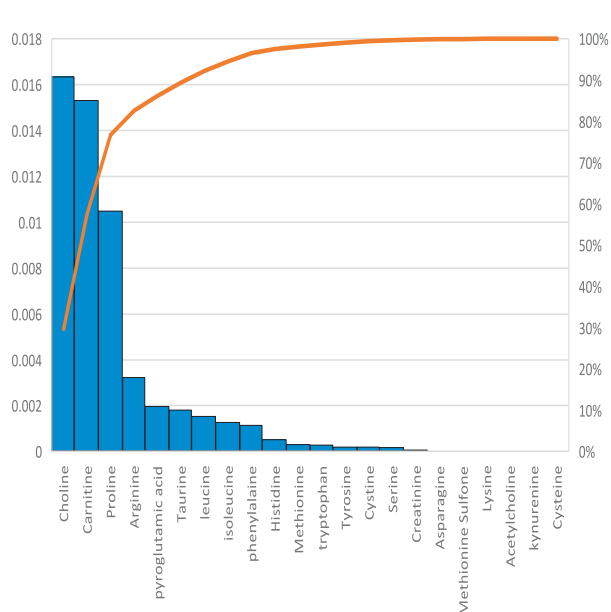


Figure 5: Effect of agrochemicals on Acetylcholine

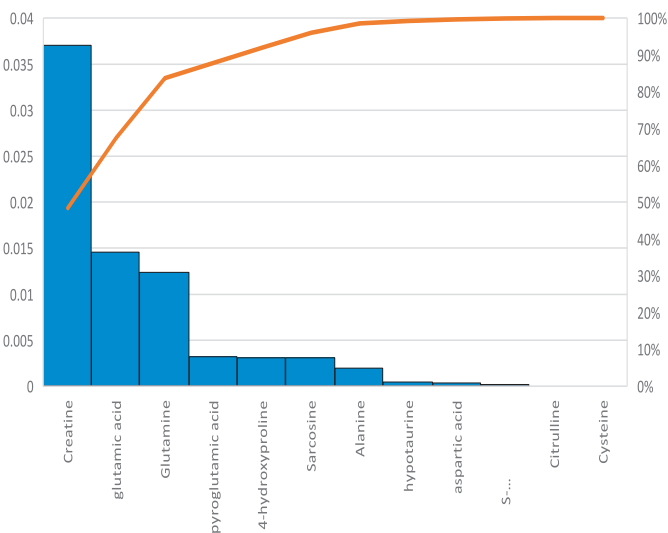
Choline, Carnitine and Proline predominant

Chloropyrifos Exposure



Creatinine, glutamic acid and Glutamine were predominant

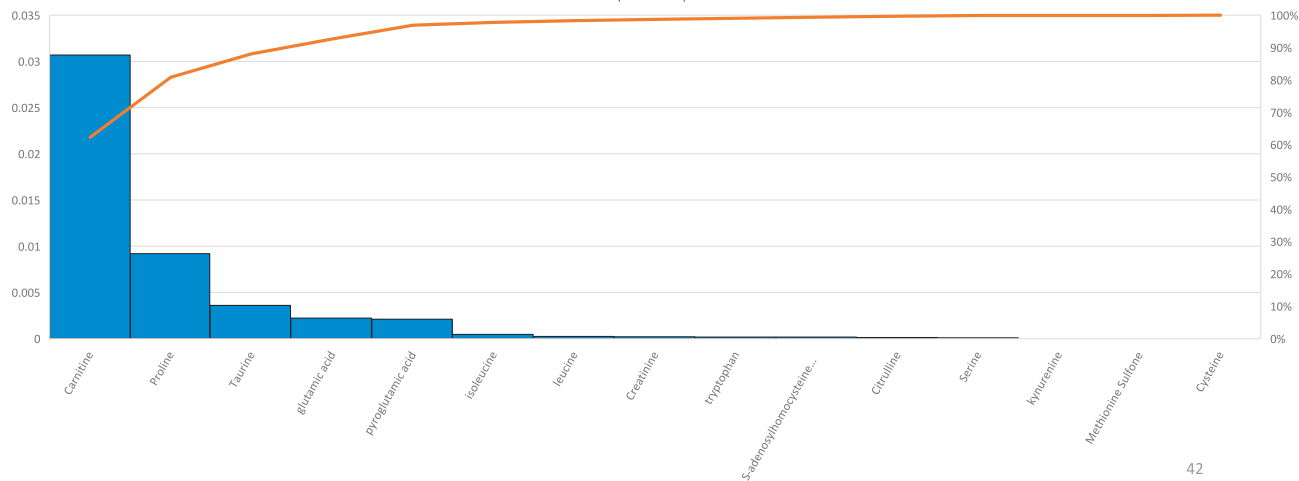
Atrazine Exposure



41

Figure 6: Effect of agrochemicals on some amino acids

Paraquat exposure



42

Figure 7: Effect of Paraquat dichloride on amino acids

Conclusion and Recommendation

Metabolomics offers great insight in the mechanism by which agrochemicals may influence changes in breast cell development thereby resulting in pathological conditions especially breast cancer. The role of amino acid metabolism in development of common pathological conditions of the breast may also

provide key biomarkers for both diagnosis and therapeutic interventions. The response of MCF10A model of normal breast cell lines to agrochemicals exposure is key for a better understanding of the effect of exposure period in relation to disease progression. The finding that agrochemicals have some effects on breast cell lines when exposed over a period is an indication

that early detection of breast cancer is key for prevention and success rate in therapeutic interventions. As we navigate the intricacies of breast cancer development, sustained efforts in research and development remains imperative to harness the full diagnostic and therapeutic potentials of metabolites such as choline, carnitine, proline and branched chain amino acids. Metabolites associated with reduction and oxidation reactions in cells are associated with pathways for the transformation of normal breast cells to breast cancer. Improved knowledge on risk associated with exposure to commonly used agrochemicals in Nigeria, prevention approaches, diagnosis and treatment is pertinent in the fight against Breast cancer.

Despite the use of very low dose of agrochemicals, changes were observed in our work, which suggests that these chemicals may cause significant modulation of protein metabolism in line with this. More experimental work is needed to measure DNA damage markers. This will be necessary to help confirm if agrochemicals inhibit cellular transcription (RNA synthesis). Further studies are needed using Multiomics approach which allows for use of integrated genomics, transcriptomics and proteomics platforms for a clearer insight in the entire component of cells. In addition, a larger cohort study may provide more insights on alterations in breast cell lines. Precautionary measures for agrochemical usage should be revisited, while formulating policies for safe agrochemical handling be prioritized together with awareness and educational workshops on agrochemical handling be considered.

References

- Cala, M. P., Aldana, J., Medina, J., Sánchez, J., Guio, J., Wist, J., & Meesters, R. J. W. (2019). Multiplatform plasma metabolic and lipid fingerprinting of breast cancer: A pilot control-case study in Colombian Hispanic women. *PLoS ONE*; **13**(2): <https://doi.org/10.1371/journal.pone.0190958>
- Chen, J., Cui, L., Lu, S., & Xu, S. (2024). Amino acid metabolism in tumor biology and therapy. *Cell Death & Disease*; **15**(1): 42. <https://doi.org/10.1038/s41419-024-06435-w>
- Chen, Z., Li, Z., Li, H., & Jiang, Y. (2019). Metabolomics: A promising diagnostic and therapeutic implement for breast cancer. In *Oncotargets and Therapy*; **12**: <https://doi.org/10.2147/OTT.S215628>
- Ekwempu, A. (2019). Safe Chemical Handling by Agrochemical Users in Plateau State, Nigeria. In *ProQuest Dissertations and Theses*.
- Fitzmaurice, C., Akinyemiju, T. F., Al Lami, F. H., Alam, T., Alizadeh-Navaei, R., Allen, C., Alsharif, U., Alvis-Guzman, N., Amini, E., Anderson, B. O., Aremu, O., Artaman, A., Asgedom, S. W., Assadi, R., Atey, T. M. H., Avila-Burgos, L., Awasthi, A., Saleem, H. O., Barac, A., ... Naghavi, M. (2018). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016 a systematic analysis for the global burden of disease study global burden of disease cancer collaboration. *Journal of American Medical Association Oncology*; **4**(11): <https://doi.org/10.1001/jamaoncol.2018.2706>
- Impouma, B., Roelens, M., Williams, G. S., Flahault, A., Codeço, C. T., Moussana, F., Farham, B., Hamblion, E. L., Mboussou, F., & Keiser, O. (2020). Measuring timeliness of outbreak response in the World Health Organization African Region, 2017–2019. In *Emerging Infectious Diseases*; **26**(11): <https://doi.org/10.3201/EID2611.191766>
- Ngrid, I., Edenfalk, H., Idong, Y., Hen, C., Admacher, I. R., Aul, P., Eltzer, M., Usterson, A. G., Anel, M., Steller, E., Allioniemi, L.-P. K., Ke, Å., Org, B., Effrey, J., & Rent, T. (2001). The New England Journal of Medicine. Gene-Expression Profiles In Hereditary Breast Cancer A Bstract Background Many cases of hereditary breast can. *New England Jjournal of Medicine*; **344**:8: <http://www.nejm.org>.
- Nittoli, A. C., Costantini, S., Sorice, A., Capone, F., Ciarcia, R., Marzocco, S., Budillon, A., & Severino, L. (2018). Effects of α -zearalenol on the metabolome of two breast cancer cell lines by 1H-NMR approach. *Metabolomics*; **14**(3): 33. <https://doi.org/10.1007/s11306-018-1330-3>
- Nolasco, D. M., Mendes, M. P. R., Marciano, L. P. de A., Costa, L. F., Macedo, A. N. De, Sakakibara, I. M., Silvério, A. C. P., Paiva, M. J. N., & André, L. C. (2023). An Exploratory Study of the Metabolite

- Profiling from Pesticides Exposed Workers. *Metabolites*; **13**(5): 596. <https://doi.org/10.3390/metabo13050596>
- Subramani, R., Poudel, S., Smith, K. D., Estrada, A., & Lakshmanaswamy, R. (2022). Metabolomics of Breast Cancer: A Review. *Metabolites*; **12**(7): 643. <https://doi.org/10.3390/metabo12070643>
- Udoh, G. D., & Gibbs, J. L. (2022). Commentary: Highlighting the need for pesticides safety training in Nigeria: A survey of farm households in Rivers State. *Frontiers in Public Health*; **10**: <https://doi.org/10.3389/fpubh.2022.988855>
- Wan, L., Liu, Q., Liang, D., Guo, Y., Liu, G., Ren, J., He, Y., & Shan, B. (2021). Circulating Tumor Cell and Metabolites as Novel Biomarkers for Early-Stage Lung Cancer Diagnosis. *Frontiers in Oncology*; **11**: <https://doi.org/10.3389/fonc.2021.630672>
- Willmann, L., Schlimpert, M., Hirschfeld, M., Erbes, T., Neubauer, H., Stickeler, E., & Kammerer, B. (2016). Alterations of the exo- and endometabolite profiles in breast cancer cell lines: A mass spectrometry-based metabolomics approach. *Analytica Chimica Acta*; **925**: <https://doi.org/10.1016/j.aca.2016.04.047>.
- Wimalawansa, S. A. and Wimalawansa, J. (2014). Agrochemical-Related Environmental Pollution: Effects on Human Health. *Global Journal of Biology, Agriculture and Health Sciences*; **3**(3): 72-83.
- Yang, L., Wang, Y., Cai, H., Wang, S., Shen, Y., & Ke, C. (2020). Application of metabolomics in the diagnosis of breast cancer: A systematic review. In *Journal of Cancer*; **11**(9): <https://doi.org/10.7150/jca.37604>.

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