Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1821-1826 | ISSN:2251-6727

Central Energy Metabolism of MCF-10A Cells Following Agrochemical Exposure: Metabolomics Approach

Ekwempu, A.I.[1], Oluboyo, A.O.[2], Odewusi, O[2]., Lorenzi, P.L.[3]
[1]Lecturer, Department of Medical Laboratory Science, Faculty of Health Science and Technology, University of Jos, Plateau State, Nigeria.
[2] Professor, Department of Medical Laboratory Science, Faculty of Medicine and Health Science, College of Medicine, ABUAD, Ekiti State, Nigeria.
[3] Professor, Faculty of Medicine, University of Texas, Houston, USA.

(Received: 16 September 2024 Revised: 11 October 2024 Accepted: 11 December 2024)

KEYWORDS

Agrochemicals, metabolomics, metabolites

ABSTRACT:
Background

Metabolomics is the study of small molecules that mediate and modulate the host of biochemical reactions that occur in all cells. Recent advances in metabolomics enable the measurement of thousands of metabolites (including polar metabolites, fatty acids, and lipids) per biological sample including those derived from the microbiome, lifestyle choices (e.g., exercise), or environmental factors (e.g. food, drugs, and chemicals). Agricultural activities in Nigeria use agrochemicals that could modulate the metabolome. 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 central energy metabolism.

Materials and Method

We treated the non- malignant breast cell line MCF10A with pesticide concentrations that are permitted in drinking water and analyzed the resulting changes in the metabolome. Our initial focus was on central energy metabolism pathways including glycolysis, the TCA cycle, pentose phosphate pathway (PPP), and nucleotide metabolism measured by ion chromatography-mass spectrometry (IC-MS) using established methodology.

Results

The low drinking water-permitted concentrations allowed, Chlorpyrifos exposure decreased the levels of several glycolysis metabolites (2,3-diphosphoglycerate, 2-phosphoglycerate, fructose 1,6-bisphosphate, phosphoenolpyruvate), pentose phosphate pathway metabolites (6-phosphogluconate, sedoheptulose 7-phosphate, NADPH), and TCA cycle metabolites (a-KG, 2-hydroxyglutarate, NADH). Simultaneously, we observed an increase in the levels of a few glycolysis metabolites (glucose 6-phosphate, fructose 2,6-bisphosphate) and pentose phosphate pathway metabolites (ribose 1-phosphate).

Atrazine also decreased the levels of several glycolysis metabolites (fructose 1,6-bisphosphate), pentose phosphate pathway metabolites (6-phosphogluconate, NADPH), and TCA cycle metabolites (2-hydroxyglutarate, NADH). Atrazine also increased the level of a few glycolysis metabolites (glucose 1-phosphate, fructose 2,6-bisphosphate) and pentose phosphate pathway metabolites (ribose 1-phosphate).

Paraquat decreased the level of several glycolysis metabolites (fructose 1,6-bisphosphate, 2-phosphoglycerate, phosphoenolpyruvate), pentose phosphate pathway metabolites (6-phosphogluconate, sedoheptulose 7-phosphate, NADPH), and TCA cycle metabolites (a-KG, 2-hydroxyglutarate, NADH). Paraquat also increased the levels of a few glycolysis metabolites (glucose 1-phosphate, glucose 6-phosphate, fructose 2,6-bisphosphate).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 1821-1826 | ISSN:2251-6727

Discussion and Conclusion

The results suggest that agrochemical exposure positively modulates breast cancer by decreasing the production of key antioxidants NADH, NADPH, and possibly also glutathione (GSH). Chlorpyrifos exposure led to the greatest decrease in production of these key antioxidants. Biochemically, decreased antioxidant capacity can give rise to higher levels of reactive oxygen species (ROS) and subsequent mutagenesis that could potentially give rise to cancer. This is also in line with various research findings that reported alterations in antioxidants ie low levels results in decreased cellular capacity to protect cells from ROS-induced damage. To further test this hypothesis, future studies will be needed to measure DNA damage markers (e.g., gamma-H2AX).

INTRODUCTION

In Nigeria, agrochemicals are commonly used by farmers for agricultural purposes such as weed control, pest control, and improvement in farm produce. The 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 increasing in Africa(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 awide range of functionsalso known as metabolome. The message in the DNA (genome) of an organism are transcribed into RNA (transcriptome), translated into protein (proteome) and finally results inthe formation of small molecules (result of metabolism) known as metabolite(Wan et al., 2021; Yang et al., 2020)s (metabolome). This implies that any change in the gene, be it mutation, over-expression, orunder-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/BRAC2genes 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., 13 C labelled glucose), volatomic (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

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1821-1826 | ISSN:2251-6727

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 central energy 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 (Ethical Clearance number: ABUADHREC/25/05/2023/193). This study design was set up to help establish a better understanding of the biological pathway of selected agrochemicals in the energy metabolism. 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. Lines. Our initial focus was on central energy metabolism pathways including glycolysis, the TCA cycle, pentose phosphate pathway (PPP), and nucleotide metabolism measured by ion chromatography-mass spectrometry (IC-MS) using established methodology. After preparation, cell components were analyzed by ultra-performance liquid chromatography (Shimadzu®, Tokyo, Japan) coupled with a mass spectrometer (Bruker®, Billerica, MA, USA). For chromatographic separation, a C_{18} reversed-phase column (100 mm × 2.1 mm 1.8 µm particle) was used, and temperature was maintained at 40 °C.

Energy metabolism pathways was determined using and based on the manufacturer's instructions(Horvath et al., *FASEB Journal*, 2017).Methods of choice were ion chromatography-mass spectrometry (IC-MS) as described in PMID: 39333384.

Statistical analysis was carried out using MetaboAnalyst 6.0 a web-based platform dedicated for comprehensive metabolomics data analysis.

Table 1: Metadata showing time points and treatment group.

Time points	Atrazine	Chlorpyrifos	Paraquat	Vehicle	Total
0days(baseline)	3	3	3	3	12
1days	3	3	3	3	12
2days	3	3	3	3	12
Grand Total	9	9	9	9	36

RESULTS AND DISCUSSION

Strikingly and unexpectedly, at the low drinking water-permitted concentrations allowed, Chlorpyrifos exposure decreased the levels of several glycolysis metabolites (2,3-diphosphoglycerate, 2-phosphoglycerate, fructose 1,6-bisphosphate, phosphoenolpyruvate) as shown in figure 1. For the pentose phosphate pathway metabolites , figure 2 shows that (6-phosphogluconate, sedoheptulose 7-phosphate, NADPH) were also decreased by chlorpyrifos exposure together with the TCA cycle metabolites (a-KG, 2-hydroxyglutarate, NADH in figure 3). Simultaneously, we observed an increase in the levels of a few glycolysis metabolites (glucose 6-phosphate, fructose 2,6-bisphosphate) and pentose phosphate pathway metabolites (ribose 1-phosphate).

Atrazine also decreased the levels of several glycolysis metabolites (fructose 1,6-bisphosphate), pentose phosphate pathway metabolites (6-phosphogluconate,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1821-1826 | ISSN:2251-6727

NADPH), and TCA cycle metabolites (2-hydroxyglutarate, NADH). Atrazine also increased the level of a few glycolysis metabolites (glucose 1-phosphate, fructose 2,6-bisphosphate) and pentose phosphate pathway metabolites (ribose 1-phosphate). This work is inline with that of *Sasikala et al.*, 2023 who showed an increased levels in Biochemical profiles in patient exposed to pesticides for a period of time

Paraquat decreased the level of several glycolysis metabolites (fructose 1,6-bisphosphate, 2-phosphoglycerate, phosphoenolpyruvate), pentose phosphate pathway metabolites (6-phosphogluconate, sedoheptulose 7-phosphate, NADPH), and TCA cycle metabolites (a-KG, 2-hydroxyglutarate, NADH). Paraquat also increased the levels of a few glycolysis metabolites (glucose 1-phosphate, glucose 6-phosphate, fructose 2,6-bisphosphate).

All 3 agrochemicals increase the level of ADP-ribose 2-phosphate and ADP-ribose, as seen in figure 4, this may reflect increased activity of TRPT1 (tRNA 2'-phosphotransferase 1) and PARG (poly-ADP-ribose glycohydrolase). These furthermore suggest elevated PARP activity; NAD+ is consumed by PARP during DNA damage repair, and PARG is activated to

regenerate NAD+ to maintain homeostasis. Together, these observatiosn suggest that agrochemical exposure causes DNA damage and activates DNA damage repair. In general, analyzing all the results of this study, we can infer that exposure to selected agrochemicals produces toxicity through multiple mechanisms, mainly through oxidative stress and mitochondrial dysfunction. This is similar to the work done by Nolasco *et al*. (2023),in an exploratory study of metabolite profiling of pesticides exposed worker.

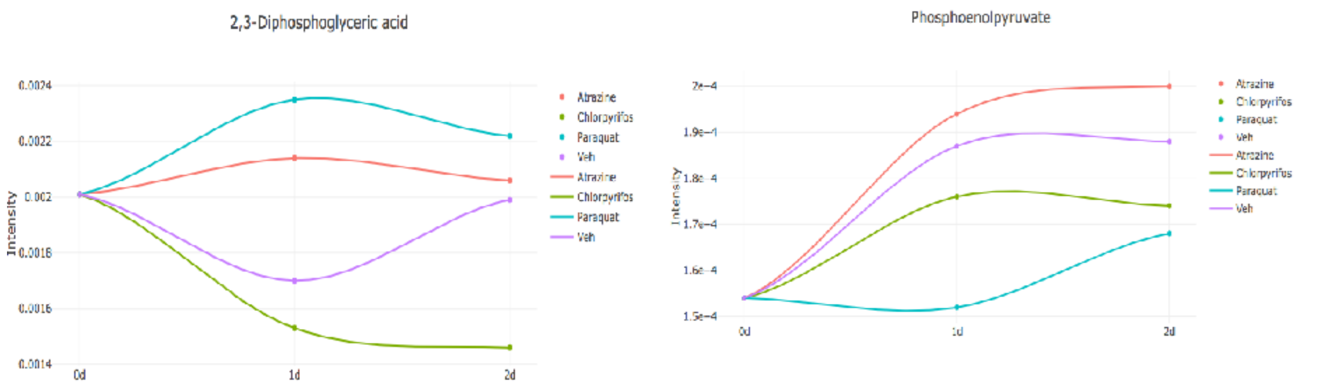

Figure 1: Effects of agrochemicals on glycolysis metabolites

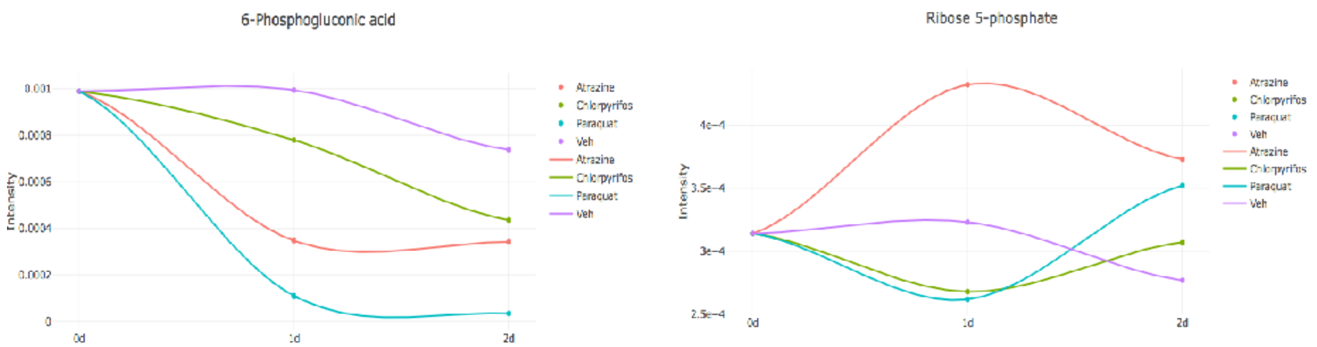

Figure 5: Metabolites of Pentode Phosphate Pathway

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1821-1826 | ISSN:2251-6727

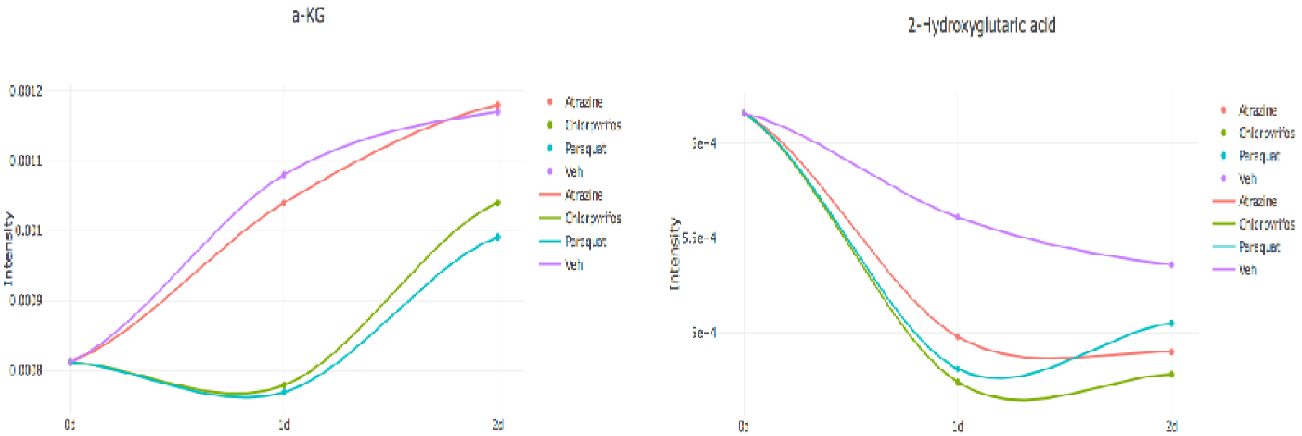

Figure 3 : Effects of Agrochemicals on TCA Pathway

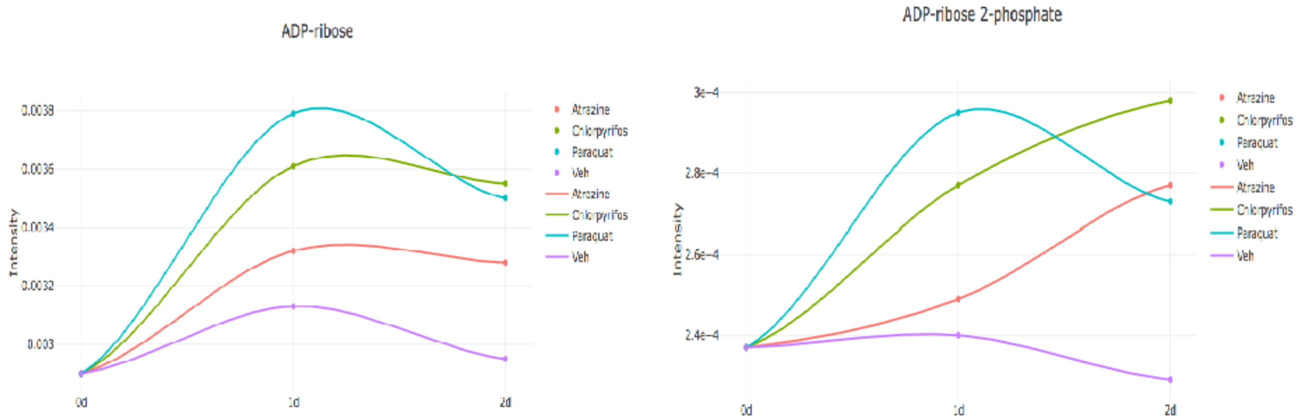

Figure 4:Effects of Agrochemicals on Nucleotide Metabolism

CONCLUSION AND RECOMMENDATIONS

Using the MCF10A cell line as a model of normal breast cells, we observed consistent effects across all three agrochemicals tested at the low doses permitted in drinking water. The results suggest that agrochemical exposure positively modulates breast cancer by decreasing the production of key antioxidants NADH, NADPH, and possibly also glutathione (GSH). Chlopyrifos exposure led to the greatest decrease in production of these key antioxidants. Biochemically, decreased antioxidant capacity can give rise to higher levels of reactive oxygen species (ROS) and subsequent mutagenesis that could potentially give rise to cancer. This is also in line with various research findings that reported alterations in antioxidants ie low levels results in decreased cellular capacity to protect cells from ROS-induced damage. To further test this hypothesis, future studies will be needed to measure DNA damage markers (e.g., gamma-H2AX).

REFERENCES

1. 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
2. Chen, Z., Li, Z., Li, H., & Jiang, Y. (2019). Metabolomics: A promising diagnostic and therapeutic implement for breast cancer. In *OncoTargets and Therapy* (Vol. 12). https://doi.org/10.2147/OTT.S215628

3. Ekwempu, A. (2019). Safe Chemical Handling by Agrochemical Users in Plateau State, Nigeria. In *ProQuest Dissertations and Theses*.
4. 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. *JAMA Oncology*, *4*(11). https://doi.org/10.1001/jamaoncol.2018.2706
5. 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* (Vol. 26, Issue 11). https://doi.org/10.3201/EID2611.191766
6. 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. In *N Engl J Med* (Vol. 344, Issue 8). http://www.nejm.org
7. 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
8. 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
9. 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
10. 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
11. 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
12. 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
13. Wimalawansa, S. A., & Wimalawansa, J. (2014). Agrochemical-Related Environmental Pollution: Effects on Human Health. *Global Journal of Biology, Agriculture and Health Sciences*, *3*(3).
14. 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* (Vol. 11, Issue 9). https://doi.org/10.7150/jca.37604