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Research Article

Atomic Absorption Spectroscopic Analysis of Heavy Metals in Cancerous Breast Tissues Among Women in Jos, Nigeria

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Abstract

Breast cancer is prevalent in northern Nigerian women most especially Jos, Plateau State owing to anthropogenic activities such as solid earth mineral mining. In this study, Atomic Absorption Spectrometry was used to determine the levels of eight heavy metals (Cd, As, Cr, Cu, Fe, Pb, Ni and Zn) in cancerous and non-cancerous breast tissues of Jos Nigerian Women. The concentration of heavy metals ranged from 1.08 to 29.34 mg/kg, 0.29 to 10.76 mg/kg, 0.35 to 51.93 mg/kg, 5.15 to 62.93 mg/kg, 11.64 to 51.10 mg/kg, 0.42 to 83.16 mg/kg, 2.08 to 43.07 mg/kg and 1.67 to 71.53 mg/kg for Cd, As, Cr, Cu, Fe, Pb, Ni and Zn respectively. Using MATLAB R2016a, significant differences ($t_v = 0.0041 – 0.0317$) existed between the levels of all the heavy metals in cancerous and non-cancerous breast tissues except Fe. At 0.01 level of significance, positive significant correlation existed between Pb and Fe, Pb and Cu, Pb and Fe, Ni and Fe, Cr and Pb, as well as Ni and Cr ($r = 0.583 – 0.998$) in cancerous breast tissues. Using ANOVA, significant differences also occurred in the levels of these heavy metals in cancerous breast tissues ($p = 1.910510 \times 10^{-26}$). The relatively high levels of the cancer-induced heavy metals (Cd, As, Cr and Pb) compared with control indicated contamination or exposure to heavy metals which could be the major cause of cancer in these female subjects.

Keywords

Atomic Absorption Spectroscopy, Breast Cancer, Heavy Metal Exposure

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1. Introduction

Women were most likely to be diagnosed with breast cancer. Annually, 2.1 million women are affected. Furthermore, it is responsible for 15% of all cancer-related deaths among women. In 2018, more than 620,000 women perished from breast cancer [10]. Although breast cancer is more prevalent in high- and upper-middle-income countries, its prevalence is increasing worldwide. Breast cancer is the most common type of cancer among women in Nigeria, a lower-middle-income country, accounting for 22.7% of all new cancer cases. In 2018, it had the highest breast cancer mortality rate among all nations, with 12,000 reported fatalities [7]. There are various distinctions between Caucasian women and women of African descent. Triple-negative cases of breast cancer are more prevalent in the Nigerian community than in those of European descent [20]. In some regions of Africa, contrary to less than 50% in Europe, more than 70% of breast cancer patients receive their diagnosis in stages 3 or 4. Data shows that premenopausal women and younger age groups are more likely to be afflicted by breast cancer in sub-Saharan Africa than in Western countries [20]. This disease has a major impact on mortality in Africa, with Nigeria having the highest age-standardized mortality rate [1].

Breast cancer is the most prevalent malignancy in women in Nigeria especially in Jos, a lower-middle-income nation, accounting for 22.7% of all new cancer cases [2]. It also has the highest breast cancer mortality rate of any country, with 12,000 deaths in 2018. Therefore, heavy metals such as Cadmium, Lead, Nickel, Chromium, Copper, Zinc, Arsenic and Iron which are known to be carcinogenic, will be analyzed in the cancerous breast tissues of women in Jos, Nigeria.

Environmental variables may play a role in the development of breast cancer, although their precise causes are not well understood but heavy metal toxicity has been linked to a high risk of breast cancer [5]. Breast cancer is a particular type of cancer that develops from the aberrant proliferation of cells in breast tissue and is marked by heterogeneity. Invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and less common inflammatory breast cancer (IBC) are some of the different ways the illness can present itself [8]. IDC and ILC accounted for 80% and 10-15% of all incidences of breast cancer, respectively. On the other hand, makes for less than 5% of cases and is a rare and aggressive type of breast cancer [18]. Genetic, hormonal, and environmental variables interact intricately to cause breast cancer. Age, sex, family history, early menstruation, late menopause, hormone replacement treatment and alcohol consumption have all been recognized as risk factors [15]. These variables are thought to disturb the natural balance of cell growth and death pathways in breast tissue while the precise processes by which they cause the development of breast cancer are not entirely understood [8].

Women working in industries with high exposure to heavy metals, such as mining, smelting, and battery manufacturing, may be more susceptible to breast cancer [16]. According to research, women working in these professions are more likely to develop breast cancer than the general population. There is growing concern about the potential health effects of extended exposure to these hazardous elements, although there is currently little evidence connecting heavy metal exposure to breast cancer [14].

2. Materials and Methods

Participants for this research were recruited from The Potter's Specialist Hospitals, Jos, on the basis of a confirmed diagnosis and scheduled for mastectomy. Following the due process as stipulated by the ethical clearance form, twenty-five cancerous and six non-cancerous breast tissues were collected after filling the consent forms. Following fixation, the tissues were washed several times before being embedded in paraffin wax. The paraffin embedding offered stability and made it easier to cut tissues into tiny slices for microscopic analysis before samples were eventually stored in collector bottles. The samples were digested using aqua regia (digestion technique with a 3:1 ratio of hydrochloric (HCl) and nitric (HNO_3) acids.

$$3HCl + HNO_3 \rightarrow 2H_2O + NOCl + Cl_2$$

The mixture was heated at 300 ℃ for 24 hrs. Deionized water was used to measure the samples after the organic materials had been entirely eliminated.

The samples were analyzed by Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer (AAS) at Pharmacology Department of Gombe State University. It is a flame AAS exciting the metallic ions in the samples at $2300\,^{o}C$. For precision and accuracy, the equipment was calibrated and the measurement was taken in triplicates following a spike. Spike additions were carried out at the midpoint of the calibration curves to give a noticeable signal owing to differences in concentration. AAS was calibrated using the solution 'blank solution' and the baseline absorption was determined. Standards were prepared for each heavy metal and the high correlation coefficients of the absorbance against concentration (R = 0.998 – 0.999) indicated the precision and accuracy of the results presented in this study.

3. Results and Discussions

Table 1 presented the descriptive statistics of levels of heavy metals in cancerous and non-cancerous breast tissues of the subjects while frequency distribution of heavy metals in cancerous and non-cancerous breast tissues.

Table 1. *Levels of heavy metals in cancerous and non-cancerous breast tissues of the subjects.*

Elements	Cancerous breast tissues (n = 25)		Non-cancerous breast tissues (n = 6)	
	Range (mg/kg)	Mean ±SD	Range (mg/kg)	Mean ±SD
Cd	4.02 - 29.34	11.75 ±1.22	1.08 - 4.92	2.94 ±0.58
As	0.75 - 10.76	4.67 ±0.50	0.29 -1.60	1.05 ±0.19
Cr	1.92 - 51.93	15.28 ±2.61	0.35 - 1.75	0.99 ±0.22
Cu	17.06 - 62.93	30.53 ±2.39	5.15 - 8.93	7.45 ±0.66
Fe	11.64 - 42.22	20.39 ±1.63	38.10 - 51.10	47.27 ±6.00
Pb	22.85 - 83.16	38.68 ±3.24	0.42 - 1.74	0.78 ±0.20
Ni	11.85 - 43.07	20.04 ±1.67	2.08 - 5.61	3.93 ±0.66
Zn	19.54 - 71.53	33.12 ±2.75	1.67 - 9.11	3.92 ±1.30

Four (As, Cd, Cr and Ni) out of the eight heavy metals considered in this research are classified by IARC (2004) as group 1 carcinogens. The levels of these heavy metals in cancerous breast tissues ranged from 4.02 to 29.4 mg/kg, 0.75 to 10.76 mg/kg, 1.92 to 51.92 mg/kg, 17.06 to 62.93 mg/kg, 11.64 to 42.22 mg/kg, 22.85 to 83.16 mg/kg, 11.85 to 43.07 mg/kg and 19.54 to 71.53 mg/kg for Cd, As, Cr, Cu, Fe, Pb, Ni and Zn respectively. Out of all the twenty-five (25) cancerous breast tissues, Pb has the highest mean concentration (38.68 mg/kg) while Arsenic had the lowest (4.67 mg/kg). The levels of heavy metals in non-cancerous breast tissues ranged from 1.08 to 4.92 mg/kg, 0.29 to 1.60 mg/kg, 0.35 to 1.75 mg/kg, 5.51 to 8.93 mg/kg, 38.10 to 51.10 mg/kg, 0.42 to 1.74 mg/kg, 2.05 to 5.61 mg/kg and 1.67 to 9.11 mg/kg for Cd, As, Cr, Cu, Fe, Pb, Ni and Zn respectively. Out of the six (6) non-cancerous breast tissues, Fe has the highest mean concentration (11.17 mg/kg) while Pb has the lowest (0.78 mg/kg). The levels of heavy metals (Cd, As, Cu, Pb, Zi and Zn) were relatively lower in non-cancerous breast tissues than the cancerous breast tissues except for Fe which is as phenomenon known as "iron addiction" nature of cancerous breast cell which makes cancer cells to have an increased need for iron for proliferation and are far more vulnerable to iron depletion than non-cancer cells [11].

Figure 1 gives the comparison of the mean concentration of heavy metals in both cancerous and non-cancerous women breast tissues. Using t-test, findings from this work revealed that the levels of these metals were relatively higher in cancerous breast tissues than non-cancerous breast tissues ($t < 0.05$). The subjects considered were observed to have been exposed to these heavy metals released into the environment as a result of the mining activities in towns or villages in the neighborhood of Plateau State, Nigeria. These metals might have found their ways through dermal contact, ingestion and inhalation. T-test was carried out to determine if significant difference exists between the levels of each heavy metals in cancerous breast and non-cancerous breast tissues. Table 2 depicts the t-test result.

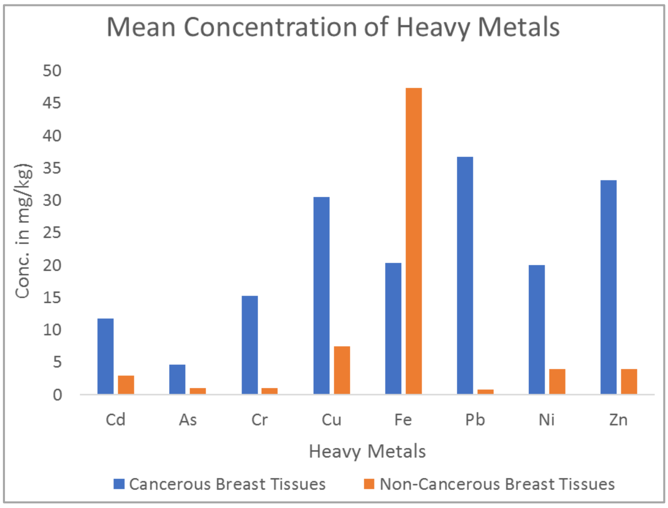

Figure 1. *Frequency distribution of heavy metals in cancerous and non-cancerous breast tissues.*

Table 2. T-test results.

Element	t-value	Remark
Cd	0.0307	S
As	0.0273	S
Cr	0.0063	S
Cu	0.0273	S
Fe	0.0571	NS
Pb	0.0317	S
Ni	0.0041	S
Zn	0.0139	S

Note: S = Significant ($t < 0.05$), NS = Not Significant ($t \geq 0.05$)

Cadmium (Cd) in cancerous breast tissues was found to have a mean concentration of 11.75 ±1.22 mg/kg compared to 2.94 ±0.58 mg/kg for non-cancerous breast tissues. Concentration of Cd was found to be relatively higher in cancerous breast tissues and non-cancerous tissues. With a t-value of 0.0307 at $p < 0.05$, significant difference exists between the two groups ($p<0.05$). This may be attributed to the estrogen like effect of cadmium which aid the production and promotion of malignant tissues in breast [19]. The mean concentration of As was 4.67 ±0.50 mg/kg in cancerous breast tissues and 1.05 ±0.19 mg/kg in non-cancerous breast tissues. Significant difference existed between the levels in the malignant tissues and control (t = 0.0273). Arsenic (As) had the lowest mean concentration among all the eight metals analyzed. The relatively high concentration of Arsenic in the cancerous breast tissues reported in this study could be attributed to the carcinogenic nature of the element to humans. Arsenic, a component of rocks can be found in water and the air due to mining activities which is one of the anthropogenic activities prevalent in Jos. The observed high level in the tissues of the cancerous subjects could also be due to the consumption of contaminated environmental samples like water and vegetables [9]. The mean concentration of Chromium (Cr) was found to be 15.28 ±2.61 mg/kg in cancerous breast tissues and relatively high compared 0.99 ± 0.22 mg/kg in non-cancerous breast tissues. There was a significant difference between the concentration of Chromium in cancerous and non-cancerous breast tissues (t = 0.0063). Chromium has been linked to the development of thyroid, lungs and prostate cancer [3]. Particulate matter that is emitted into the environment by the metal industry exposes people to chromium. Contaminants in the land, water, air and diet may cause further exposure. The mean concentration of Copper (Cu) in cancerous breast tissues was found to be 30.53 ±2.39 mg/kg which was lower than that of the non-cancerous breast tissues (7.45 ±0.66 mg/kg). Significant difference also existed in the concentration of copper between cancerous breast tissues and control (t = 0.0273). Copper is an essential element in human and it can pose danger if its concentration is extremely high in human being. Copper is associated with the growth and development of new blood vessels in cancerous breast tissues, a term which is known as angiogenesis [12]. The mean concentration of Iron (Fe) in cancerous breast tissues was found to be 47.27 ±6.00 mg/kg which is lower to a concentration of 42.22 ± 11.6 mg/kg that was found in non-cancerous breast tissues. The t-value of Iron (Fe) concentration in cancerous and non-cancerous tissues is 0.0571. Iron (Fe) is an essential element for human being but it is over secretion has been linked with the occurrence and progression of cancer of the lungs. Despite this, the dependence on iron in malignant tissues increases, Fe is addicted to cancerous breast tissues [11]. It has been established that iron possesses hybrid qualities that may promote the development of cancerous tissues or promote their demise [4]. Weathered rocks, soil and water that is in contact with weathered rocks are linked to iron exposure. Lead (Pb) was found to have a mean concentration of 38.68 ± 3.24 mg/kg in cancerous breast tissues and a mean concentration of 0.78 ±0.20 in non-cancerous breast tissues. There exists a significant difference in the concentration of Lead (Pb) in both groups (t_v = 0.0317). The prevalence of Lead (Pb) is attributed to industrial activities which contributes to the occurrence and progression of breast cancer in the study area. The mean concentration of Ni in cancerous breast tissues was 20.04 ±1.67 mg/kg while that of non-cancerous breast tissues was 3.93 ±0.66 mg/kg. The t-test result indicated that (t_v = 0.0041) significant difference exists between the levels of Nickel (Ni) in both cancerous and non-cancerous breast tissues. Nickel and its compounds can be exposed by food, dermal contact and inhalation [6]. In this study, the high level of nickel discovered in the malignant breast tissues of the subjects could also be as a result of the exposure of these women to mining dust. The result obtained in this research is similar to the value reported by Buxton *et al* in 2019 where higher concentration was found in malignant breast tissues than the control group [6]. The mean concentration of Zinc in cancerous and non-cancerous breast tissues were 3.12 ±2.75 mg/kg and 3.92 ± 1.30 mg/kg respectively. Significant difference also exists between the concentration of zinc in both cancerous breast tissues and non-cancerous breast (Table 2). The result obtained here was very close to the value reported by Richter *et al in* 2017 [13]. Zinc is an essential trace element second the concentration of Iron in the body. Although, zinc is a naturally occurring element, human activities such as mining, metal smelting, coal burning and waste disposal can significantly affect the concentration of zinc. Table 3 gives the comparison of cancerous and non-cancerous breast tissues with literature values or the results obtained in similar studies.

Table 3. *Levels of heavy metals in the cancerous and non-cancerous breast tissues with literature.*

Elements	Concentration of cancerous breast tissues in this study (mg/kg)	Sarita (2012)	Mohammadi *et al* (2014)	Pasha *et al* (2008)	Mehmet *et al* (2007)
Cd	11.75		38.66	2.64	33.00
As	4.67				
Cr	15.28	10.40			
Cu	30.53	32.30			4.30
Fe	20.39				42.00
Pb	36.68		35.69	13.20	
Ni	20.04				
Zn	33.12	13.00			45.00

The Inter-Elemental Pearson Correlation among the heavy metals in cancerous breast tissues selected for this study was also carried out. The results obtained were presented in Table 4. At 0.01 level of significance, positive significant correlation existed between Pb and Fe, Pb and Cu, Pb and Fe, Ni and Fe, Cr and Pb, as well as Ni and Cr (r = 0.583 – 0.998) in cancerous breast tissues. This indicated that an increase in one could lead to an increase in the other and some could be used as pathfinders for others.

Table 4. *Pearson Correlation for heavy metals in cancerous breast tissues.*

	Cd	As	Cr	Cu	Fe	Pb	Ni	Zn
Cd	1.000	0.583**	0.442	0.337	0.281	0.287	0.288	0.275
As	0.583**	1.000	0.071	-0.004	-0.012	-0.011	-0.090	-0.020
Cr	0.442*	0.070	1.000	0.799**	0.789**	0.788**	0.787**	0.782**
Cu	0.337	-0.004	0.799**	1.000	0.982**	0.981**	0.981**	0.979**
Fe	0.281	-0.012	0.789**	0.982**	1.000	0.998**	0.998**	0.997**
Pb	0.287	-0.110	0.288**	0.982**	0.998**	1.000**	1.000**	1.000**
Ni	0.288	-0.009	0.787**	0.981**	0.998**	1.000**	1.000	1.000**
Zn	0.275	-0.020	0.782**	0.979**	0.997**	1.000**	1.000**	1.000

Note: **Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Figure 2 below is an ANOVA graph by MATLAB R2016a. An extremely low p-value of 1.910510×10^{-26} was determined in one-way ANOVA analysis. The differences between the groups that were detected are very significant and highly unlikely to have happened by chance, according to the p-value. In particular, the p-value for the malignant breast tissues was almost zero, indicating significant differences between the groups. These results suggested that the groups being compared in the one-way ANOVA do indeed differ significantly.

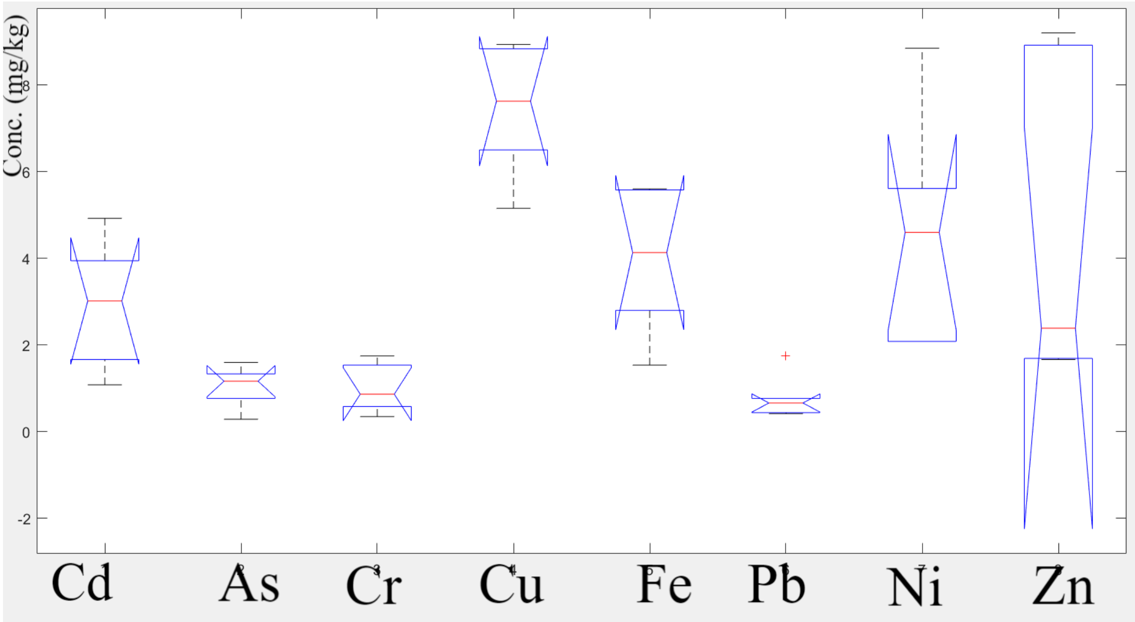

Figure 2. *ANOVA Graph of Cancerous Breast Tissues.*

Figure 3 below is a graph of ANOVA graph of non-cancerous breast tissues as computed by MATLAB R2016a. In the ANOVA analysis, a remarkably small p-value of 1.0164×10^{-6} has been obtained, which is typically considered as strong evidence to suggest that the observed differences in the group means are unlikely to have happened randomly. In simpler terms, the result also indicated that there was a high probability that significant differences exist among the group.

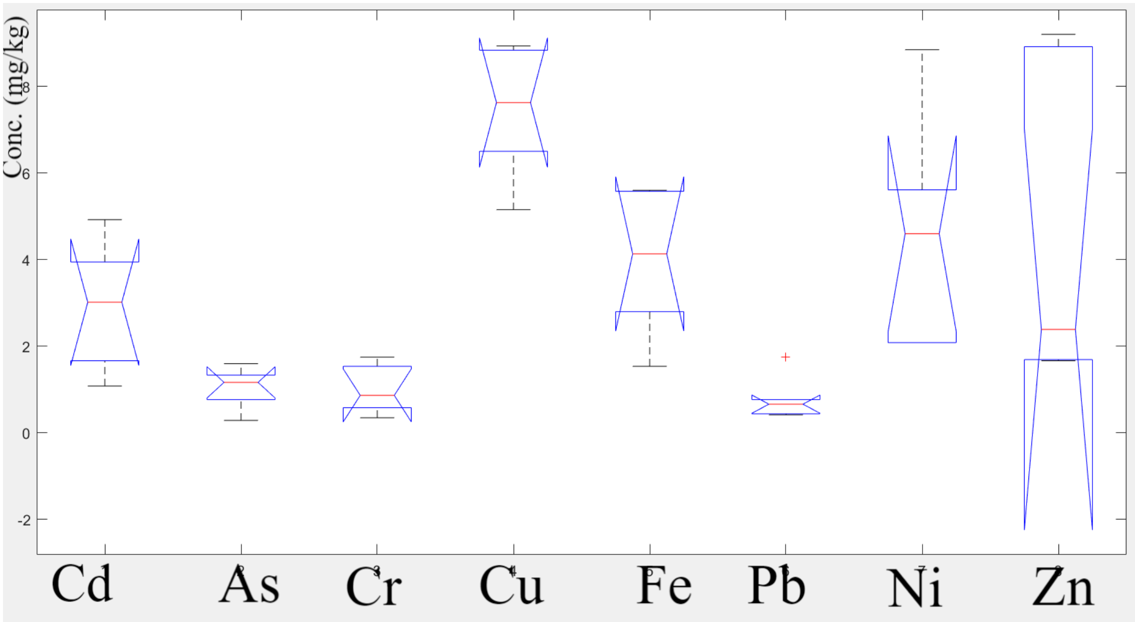

Figure 3. *ANOVA Graph for Non-cancerous Breast Tissues.*

4. Conclusion

The concentration of heavy metals (Cd, As, Cr, Cu, Fe, Pb, Ni and Zn) in the cancerous breast tissues and non-cancerous breast tissues among women in Jos, Plateau State Nigeria, have been determined using Atomic Absorption Spectroscopy. The concentration of heavy metals was within 0.75 to 83.16 mg/kg for cancerous breast tissues and 0.29 mg/kg to 51.10 for non-cancerous breast tissues. Using MATLAB, significant difference (t < 0.05) also existed between the levels of Cd, As, Cr, Cu, Pb, Ni and Zn in cancerous and non-cancerous breast tissues except for Fe. ANOVA significant differences existed in the levels of these heavy metals in the cancerous breast tissues. The mean concentration of Cd, As, Cr, Cu, Fe, Pb, Ni and Zn are 11.75 mg/kg, 4.67 mg/kg, 15.28 mg/kg, 30.53 mg/kg, 20.39 mg/kg, 36.68 mg/kg, 20.04 mg/kg and 33.12 mg/kg respectively while it was 2.94 mg/kg, 1.05 mg/kg, 0.99 mg/kg, 7.45 mg/kg, 11.17 mg/kg, 0.78 mg/kg, 3.93 mg/kg and 3.92 mg/kg respectively. The t-test result of -4.2079 between the mean concentration of cancerous and non-cancerous tissues shows that there is significant difference between the both samples. The results obtained from the quantitative analysis of this research gave a valuable insight into factors contributing to high concentration of heavy metals in in cancerous breast tissues. This was evidence of contamination as a result of exposure by ingestion, inhalation or other means to one anthropogenic activity of the other. Therapeutic measures such as gastric lavage, ascorbic acid consumption, and divalent cation treatment are all effective ways to manage heavy metal toxicity in people. It has also been discovered to be helpful to use a range of chelating drugs during the acute toxicity phase [17].

Abbreviations

IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
IBC	Inflammatory Breast Cancer
AAS	Atomic Absorption Spectrometer

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Angell, B., Sanuade, O., Adetifa, I. M. O., Okeke, I. N., Adamu, A. L., Aliyu, M. H., Ameh, E. A., Kyari, F., Gadanya, M. A., Mabayoje, D. A., Yinka-Ogunleye, A., Oni, T., Jalo, R. I., Tsiga-Ahmed, F. I., Dalglish, S. L., Abimbola, S., Colbourn, T., Onwujekwe, O., Owoaje, E. T., … Abubakar, I. (2022). Population health outcomes in Nigeria compared with other west African countries, 1998–2019: a systematic analysis for the Global Burden of Disease Study. *Lancet (London, England)*, *399*(10330), 1117. https://doi.org/10.1016/S0140-6736(21)02722-7

[2] Azubuike, S. O., Muirhead, C., Hayes, L., & McNally, R. (2018). Rising global burden of breast cancer: The case of sub-Saharan Africa (with emphasis on Nigeria) and implications for regional development: A review. *World Journal of Surgical Oncology*, *16*(1), 1–13. https://doi.org/10.1186/S12957-018-1345-2/FIGURES/7

[3] Batyrova, G., Kononets, V., Amanzholkyzy, A., Tlegenova, Z., & Umarova, G. (2022). Chromium as a Risk Factor for Breast Cancer: A Meta-Analysis. *Asian Pacific Journal of Cancer Prevention: APJCP*, *23*(12), 3993. https://doi.org/10.31557/APJCP.2022.23.12.3993

[4] Brown, R. A. M., Richardson, K. L., Kabir, T. D., Trinder, D., Ganss, R., & Leedman, P. J. (2020). Altered Iron Metabolism and Impact in Cancer Biology, Metastasis, and Immunology. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/FONC.2020.00476

[5] Büsselberg, D., & Florea, A. M. (2011). Metals and Breast Cancer: Risk Factors or Healing Agents? *Journal of Toxicology*, *2011*. https://doi.org/10.1155/2011/159619

[6] Buxton, S., Garman, E., Heim, K. E., Lyons-Darden, T., Schlekat, C. E., Taylor, M. D., & Oller, A. R. (2019). Concise Review of Nickel Human Health Toxicology and Ecotoxicology. *Inorganics 2019, Vol. 7, Page 89*, *7*(7), 89. https://doi.org/10.3390/INORGANICS7070089

[7] Fatiregun, O. A., Oluokun, T., Lasebikan, N. N., Nwachukwu, E., Ibraheem, A. A., & Olopade, O. (2021). Breast Cancer Research to Support Evidence-Based Medicine in Nigeria: A Review of the Literature. *JCO Global Oncology*, *7*, 384–390. https://doi.org/10.1200/go.20.00541

[8] Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T. C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & Diseases*, *5*(2), 77. https://doi.org/10.1016/J.GENDIS.2018.05.001

[9] Khan, S., Shah, I. A., Muhammad, S., Malik, R. N., & Shah, M. T. (2015). Arsenic and Heavy Metal Concentrations in Drinking Water in Pakistan and Risk Assessment: A Case Study. *Human and Ecological Risk Assessment*, *21*(4), 1020–1031. https://doi.org/10.1080/10807039.2014.950925

[10] Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review. *Cancers*, *13*(17). https://doi.org/10.3390/CANCERS13174287

[11] Manz, D. H., Blanchette, N. L., Paul, B. T., Torti, F. M., & Torti, S. V. (2016). Iron and cancer: recent insights. *Annals of the New York Academy of Sciences*, *1368*(1), 149. https://doi.org/10.1111/NYAS.13008

[12] Nishida, N., Yano, H., Nishida, T., Kamura, T., & Kojiro, M. (2006). Angiogenesis in Cancer. *Vascular Health and Risk Management*, *2*(3), 213. https://doi.org/10.2147/VHRM.2006.2.3.213

[13] Richter, P., Faroon, O., & Pappas, R. S. (2017). Cadmium and Cadmium/Zinc Ratios and Tobacco-Related Morbidities. *International Journal of Environmental Research and Public Health*, *14*(10). https://doi.org/10.3390/IJERPH14101154

[14] Semu, E. (2019). Heavy Metals and Organopesticides: Ecotoxicology, Health Effects and Mitigation Options with Emphasis on Sub-Saharan Africa. *Toxicology: Current Research*, *3*(1), 1–14. https://doi.org/10.24966/TCR-3735/100010

[15] Shah, N. R., & Wong, T. (2006). Current breast cancer risks of hormone replacement therapy in postmenopausal women. *Expert Opinion on Pharmacotherapy*, *7*(18), 2455. https://doi.org/10.1517/14656566.7.18.2455

[16] Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *EXS*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

[17] Umesh C. Gupta, & Subhas C. Gupta. (2016). Heavy Metal Toxicity in Humans and its Preventive and Control Measures. *Current Nutrition & Food Science*, *7*(4), 221–231. https://doi.org/10.2174/1573401311107040221

[18] Xiao, Y., Ma, D., Ruan, M., Zhao, S., Liu, X. Y., Jiang, Y. Z., & Shao, Z. M. (2017). Mixed invasive ductal and lobular carcinoma has distinct clinical features and predicts worse prognosis when stratified by estrogen receptor status. *Scientific Reports*, *7*(1). https://doi.org/10.1038/S41598-017-10789-X

[19] Yue, W., Wang, J. P., Li, Y., Fan, P., Liu, G., Zhang, N., Conaway, M., Wang, H., Korach, K. S., Bocchinfuso, W., & Santen, R. (2010). Effects of estrogen on breast cancer development: role of estrogen receptor independent mechanisms. *International Journal of Cancer. Journal International Du Cancer*, *127*(8), 1748. https://doi.org/10.1002/IJC.25207

[20] Zheng, Y., Walsh, T., Gulsuner, S., Casadei, S., Lee, M. K., Ogundiran, T. O., Ademola, A., Falusi, A. G., Adebamowo, C. A., Oluwasola, A. O., Adeoye, A., Odetunde, A., Babalola, C. P., Ojengbede, O. A., Odedina, S., Anetor, I., Wang, S., Huo, D., Yoshimatsu, T. F., … Olopade, O. I. (2018). Inherited Breast Cancer in Nigerian Women. *Journal of Clinical Oncology*, *36*(28), 2820. https://doi.org/10.1200/JCO.2018.78.3977