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(RESEARCH ARTICLE)

Biochemical determination of reduced glutathione concentration in pig liver and kidney in the presence of chlorinated aquatic humic materials

Nse Udoka Ebe [1,*], Grace Sylvester Effiong [1], Oboso Edem Etim [1], Uduak Onofiok Luke [1], Othuke Bensandy Odeghe [2] and Jessie Idongesit Ndem [1]

[1] *Department of Biochemistry, Faculty of Science, University of Uyo, Uyo, Nigeria.*
[2] *Department of Biochemistry, Faculty of Science, Madonna University, Elele, Nigeria.*

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Abstract

Disinfection of surface waters for human consumption and use often results in reaction between the disinfectant (generally chlorine) and organic substrates present at the source. These substrates are majorly in the form of aquatic humic materials. This work was designed to assess reduced glutathione (GSH) concentration in pig liver and kidney in the presence of chlorinated aquatic humic materials (CAHMs) using biochemical parameters. GSH concentration in pig liver and kidney was investigated following standard procedures using Ellman's reagent. Effect of chlorinated local and foreign aquatic humic products on the GSH concentration of the two tissues was studied in line with standard methodologies. GSH concentration was found to be higher in pig liver (0.874 ± 0.003µmol/g) than the kidney (0.545 ± 0.002µmol/g). All the chlorinated aquatic humic samples studied were found to deplete the GSH concentration of the two tissues, with the more toxic and concentrated purified chlorinated foreign aquatic humics having higher depletion. Certain amount of GSH was also found to be left in both tissues after conjugation with the chlorinated aquatic humic samples which perhaps could be used for subsequent conjugation and detoxification of water pollutants by the tissues. In conclusion, the liver contains more GSH than the kidney. The levels of this antioxidant in pig liver and kidney were both reduced by the aquatic humic materials used. This study may therefore reemphasize earlier suggestions that the amino acids needed for cellular synthesis of GSH could be supplemented regularly through dietary sources.

Keywords: Reduced glutathione; Pig liver and kidney; Chlorination; Aquatic humic materials

1. Introduction

Glutathione (GSH) is a cellular antioxidant comprising of three amino acids namely, glutamic acid, cysteine and glycine. It is a strong nucleophile which reacts well with soft electrophiles, but poorly with both weak and strong electrophiles [1]. The presence of glutathione is required to maintain the normal function of the immune system. It is known to play a critical role in the multiplication of lymphocytes (the cells that mediate specific immunity) which occurs in the development of an effective immune response. The cells of the immune system always produce many oxiradicals as a result of their normal functioning, resulting in a need for higher concentrations of antioxidants than most cells [2]. Glutathione plays a crucial role in fulfilling this requirement.

Humic materials on the other hand, are organic substances that are formed by the profound alteration of organic matter in a natural environment. In other words, humic substances are decayed products arising from the flora and fauna that live in the aquatic environment, and they may also be acquired from runoff containing decomposed natural products leached from the soil. Humic substances are complex in nature. The mode of their presence in natural waters is largely

* Corresponding author: Nse Udoka Ebe
Department of Biochemistry, Faculty of Science, University of Uyo, Uyo, Nigeria.

unknown. They are generally considered to arise from microbial degradation of organic debris such as leaves, grass, wood or animal waste and are responsible for much of the colour of surface water. They contain phenolic, quinoid, poly-carboxylic, aromatic, acidic and aliphatic groups, with the aromatic groups predominating. In addition to benzene, carboxylic acid and phenolic groups, some authors have included fatty acids, proteins and polysaccharides groups. It has been assumed that these groups are joint together covalently by linkages such as ether, ester and carbon-carbon to form three dimensional, polymers of high molecular weight as earlier documented [3] [4] [5].

At the present time, society is becoming increasingly conscious of the presence and hazards of trace amounts of toxic organic compounds which, inadvertently or otherwise, have entered the environment. Such compounds as have been noted do not share the same structural features as the bulk of the dissolved organic material but tend to be smaller and non-ionic molecules. Previous studies revealed that most abundant humic acid fractions form aggregates in solutions that are made of relatively small molecules (molecular weights of several thousand or less). The degree of association of these molecules is a function of pH [6]. It had also been reported that the degree of aggregation of some of the fractions is also a function of concentration and that at low concentrations the molecules are dissociated. The presence of these aggregates appears to have marked effect on the way humic acids interact with pollutants in natural water systems [7].

Many authors emphasized that GSH-deficient cells do not provide test systems with elevated sensitivity for the detection of mutagenic chlorinated humic substances [8][9]. The authors however maintained that anomalies exist in the effect of extracellular and intracellular GSH on the genetic toxicity of chlorinated humic substances. They added that non-linear dependency on GSH concentration may be an important factor in this regard.

Based on the above submissions, and the fact that reduced glutathione has been found to play a vital role in detoxification of pollutants in the liver; but the level of this antioxidant in the cells has not been widely studied, these however, necessitated the present work. The aim of this study is to assess reduced glutathione concentration in pig liver and kidney in the presence of chlorinated aquatic humic materials (CAHMs) using biochemical parameters.

2. Material and methods

2.1. Sample Collection and Preparation

Pig liver and kidney were purchased from Ikot Ekpene in Akwa Ibom State, Nigeria. The tissues were identified and authenticated at the Faculty of Agriculture, University of Uyo, Nigeria by Dr Ime Okon. The freshly obtained specimens were used immediately [10].

Unpurified Local Aquatic Humic Material Samples (HMSs) were randomly obtained from four (4) different drinking waters within Nigeria. Details concerning all the local humic material samples are shown in Table 1.

However, Purified Foreign Aquatic Humic Materials (FHMs) used namely, FHM-1, FHM-2, FHM-3 and FHM-4 were kindly provided by Professor G. A. Ubom of the Department of Biochemistry, University of Jos, Nigeria who obtained them from the US and British water sources and purified them accordingly.

Table 1 Name and Source of Local Aquatic Humic Material Samples

Sample	Local name	English/scientific name	Source	Location/ state
HMS-Yelwa	Malmo	Water Pear leaves (*Syzygium guineense*)	Yelwa-Zangam Pond	Jos North, Plateau
HMS-Alau	Ristata	Eucalyptus leaves (*Eucalyptus camadulensis*)	Alau Dam	Maiduguri, Borno
HMS-Oji	Achara	Bambo Leaves (*Bambusa vulgaris*)	Oji River	Oji, Enugu
HMS-Ikot Utin	Mfang Etoidim	Guinea Peach leaves (*Nauclea latifolia*)	Ikot Utin River	Essien Udim, Akwa Ibom

2.2. Treatment of Local Aquatic Humic Materials and Purified Foreign Humic Samples before Chlorination

Decayed leaves of local samples picked from the bottom of the respective ponds/rivers were sun dried for 8 days and later pounded with mortar to obtain humic material samples in powdered form. Absorbance of the derived unpurified local aquatic humic materials and the purified foreign humic samples were read at 220-440 nm [11] [12].

2.3. Chlorination of Local and Foreign Aquatic Humic Samples

All the local and foreign aquatic humic samples were chlorinated by applying standard steps [13] [14]. Here, 40mg each of the local aquatic humic materials was dissolved in 400 cm^3 of saline buffer pH 7.4 placed in separate beakers. Thereafter, 40mg of calcium hypochlorite was added. These were covered and shaken using an electric shaker for 10 hours. The beakers were later placed in fume cupboard for 1 hour to evaporate excess chlorine. Derived chlorinated local aquatic humic materials [CLHM] were further dissolved in 400 cm^3 each of saline buffer pH 7.4. The foreign aquatic humic samples were treated in like manner to obtain chlorinated foreign aquatic humic materials [CFHM]. Absorbance of the derived CLHM and CFHM samples were read at 220-440 nm [11] [12].

2.4. Determination of GSH Concentration in Pig Liver and Kidney (control)

GSH concentration in pig liver and kidney was determined following standard procedures using Ellman's reagent [15] [16]. Prior to this, the following solutions were prepared:

- 50 mM Tris-HCl buffer pH 7.4 was prepared by dissolving 7.90 g of Trizma HCl (Tris (hydroxymethyl) aminomethane hydrochloride) and made up to 1 litre with distilled water. The pH was adjusted to 7.4 with HCl or NaOH.
- 0.3 M phosphate buffer pH 7.4 containing 1mM EDTA was prepared by mixing 0.037 g of ethylene diaminetetra acetic acid (EDTA) (molecular weight 372.24), 3.51g of potassium dihydrogen phosphate (molecular weight 117) and 4.26g of disodium hydrogen phosphate (molecular weight 142) and made up to 100ml with distilled water. The pH was adjusted to 7.4 using HCl or NaOH.
- 1% Trisodium citrate solution was also prepared by dissolving 1.0g of trisodium citrate salt and made up to 100 cm^3 with distilled water.
- 0.04% of dithiobis-(2-nitrobenzoic acid) (DTNB) was prepared by dissolving 0.04 g of DTNB and made up to 100 cm^3 with 1% trisodium citrate solution.

All the prepared solutions were placed in separate containers properly labelled.

Portion of the liver and kidney (1.0g each) was homogenized separately in ice-cold 50 mM Tris-HCl buffer pH 7.4 (1/10, w/v) in a homogenizer. The homogenates were centrifuged at 2400 x g for 10 minutes and the supernatant (S_L and S_K respectively) was separated using Pasteur pipette and then used for biochemical assays.

To 1.0 cm^3 of the derived supernatant (S_L) and glutathione standard was added 8.5 cm^3 of 0.3M phosphate buffer containing 1 mM EDTA pH 7.4, and 2.0 cm^3 of a solution of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) – 0.04% (w/v) in 1% trisodium citrate. Similar treatment was also given to derived supernatant (S_K). Absorbance of the two mixtures was read at 412 nm against water blank.

GSH concentration in the two tissues was calculated using the formula:

$$\text{GSH concentration} = \frac{\text{OD test}}{\text{OD std}} \times \text{concentration of std.} \ldots\ldots\ldots (a)$$

Where;
OD = Optical density
Std. = Standard (GSH standard)

In addition, the effect of chlorinated local and foreign aquatic humic material samples on the GSH concentration of the two tissues was investigated.

2.5. Determination of GSH Concentration in Pig Liver and Kidney after Exposure to CLHM

A quantity (1.0 cm^3) of the derived supernatant (S_L) and glutathione standard was added to 8.5 cm^3 of 0.3M phosphate buffer containing 1 mM EDTA pH 7.4, and 2.0 cm^3 of a solution of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) – 0.04% (w/v) in 1% trisodium citrate and placed respectively in four (4) different beakers. Each of the chlorinated local humic

material samples (1.0 cm^3) was added respectively to each beaker and left in the refrigerator overnight. Similar procedure was carried out using derived supernatant (S_K). The absorbance of the mixtures was read at 412 nm against water blank. GSH concentration in the tissue samples was calculated using the formula in (a) above.

2.6. Determination of GSH Concentration in Pig Liver and Kidney after Exposure to CFHM

The same quantity (1.0 cm^3) of the derived supernatant (S_L) and glutathione standard was added to 8.5 cm^3 of 0.3 M phosphate buffer containing 1mM EDTA pH 7.4, and 2.0 cm^3 of a solution of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) – 0.04% (w/v) in 1% trisodium citrate and placed respectively in four (4) different beakers. Each of the chlorinated foreign humic material samples (1.0cm^3) was added respectively to each beaker and left in the refrigerator overnight. Same treatment was also performed with derived supernatant (S_K). The absorbance of the mixtures was read at 412 nm against water blank. GSH concentration in the tissue samples was calculated using the same formula indicated in (a) above.

2.7. Statistical Analysis

Data are presented as mean ± SD (standard deviation). Statistical analysis of the data was done using the statistical package for the social science software (SPSS) programme. One way analysis of variance (ANOVA) with post hoc analysis was used to assess the differences between the samples and statistical significance was considered at $p < 0.05$

3. Results and discussion

3.1. Spectroscopic Study of Local and Foreign Aquatic Humic Samples before and after Chlorination

The results of the effect of the chlorinated local and foreign aquatic humic samples on GSH concentration in pig liver and kidney are displayed in table 2 below. Similarly, results of the spectroscopic study are shown on figures 1- 16.

Table 2 Effect of Chlorinated Local and Foreign Aquatic Humic Material Samples on GSH Concentration in Pig Liver and Kidney

Samples	GSH Concentration (µmol/g tissue)	
	Liver	Kidney
Control	0.874 ± 0.003	0.545 ± 0.002
Tissue + CLHM-Yelwa	0.701 ± 0.002	0.472 ± 0.001
Tissue + CLHM-Alau	0.714 ± 0.001	0.480 ± 0.002
Tissue + CLHM-Oji	0.651 ± 0.002	0.368 ± 0.001
Tissue + CLHM-Utin	0.686 ± 0.002	0.397 ± 0.002
Tissue + CFHM-1	0.423 ± 0.002	0.201 ± 0.001
Tissue + CFHM-2	0.435 ± 0.002	0.220 ± 0.002
Tissue + CFHM-3	0.426 ± 0.001	0.213 ± 0.002
Tissue + CFHM-4	0.440 ± 0.001	0.224 ± 0.003

Values are expressed as mean ± standard deviation, n = 4; Values are significantly different from control ($p < 0.05$).

Table 2 above shows the concentrations of GSH in pig liver and kidney before and after exposure to chlorinated local and foreign aquatic humic materials. Here, GSH concentration was found to be higher in the liver than the kidney. Tissues exposed to CAHMs showed significant reduction in the concentration of GSH when compared to the control. This suggests that the chlorinated local and foreign aquatic humic material samples depleted GSH in the two tissues examined. However, the GSH concentration was significantly lower in tissues exposed to CFHMs than those with CLHMs.

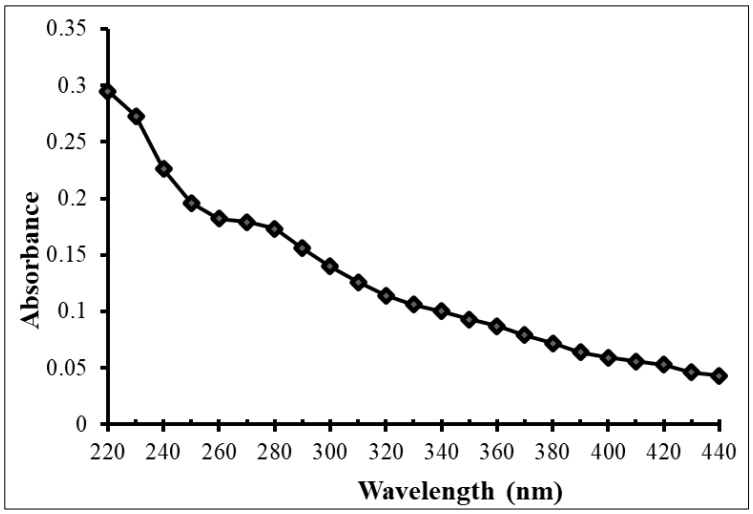

Figure 1 Absorption Spectrum of HMS-Yelwa

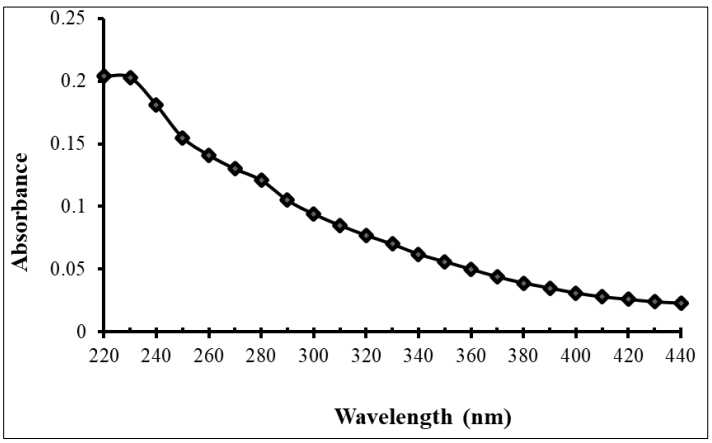

Figure 2 Absorption Spectrum of CLHM-Yelwa

Figures 1 and 2 above show the UV absorption spectra of HMS-Yelwa before and after chlorination respectively

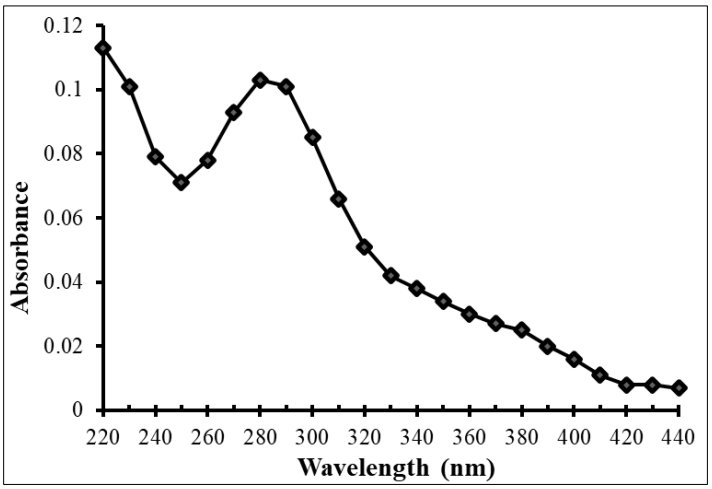

Figure 3 Absorption Spectrum of HMS- Alau

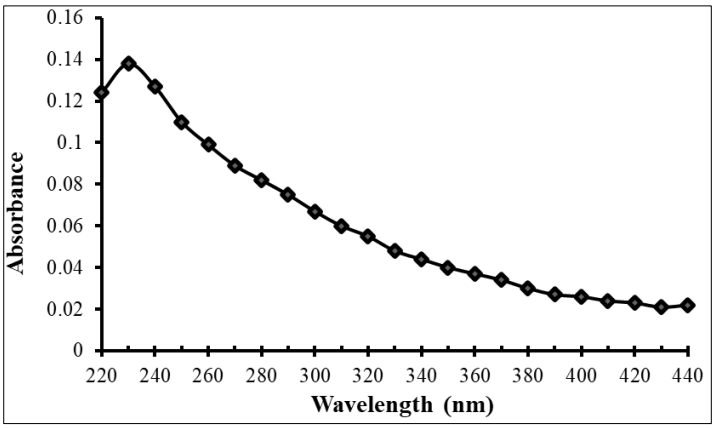

Figure 4 Absorption Spectrum of CLHM- Alau

Figures 3 and 4 above respectively show UV absorption spectrum of HMS- Alau before and after chlorination

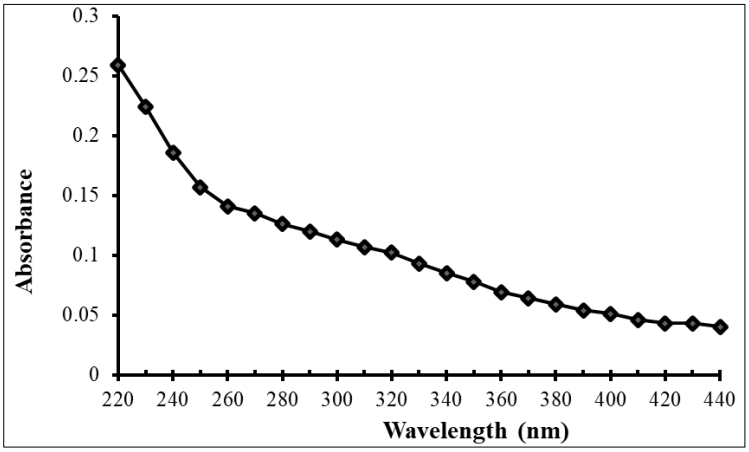

Figure 5 Absorption Spectrum of HMS- Oji

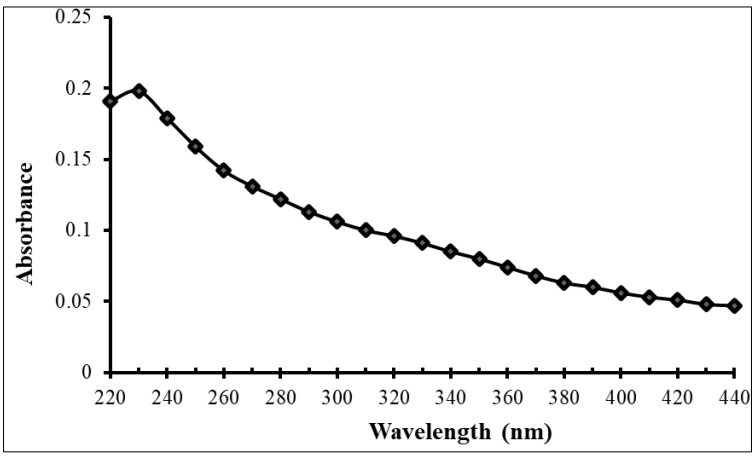

Figure 6 Absorption Spectrum of CLHM- Oji

UV absorption spectra of HMS- Oji before and after chlorination are shown on figures 5 and 6 respectively

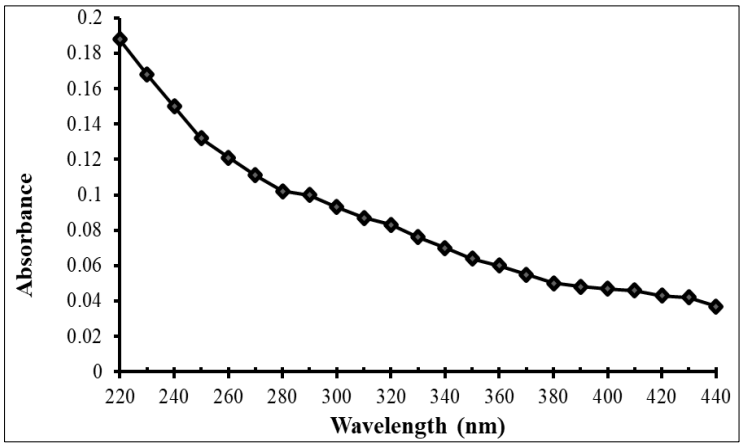

Figure 7 Absorption Spectrum of HMS- Ikot Utin

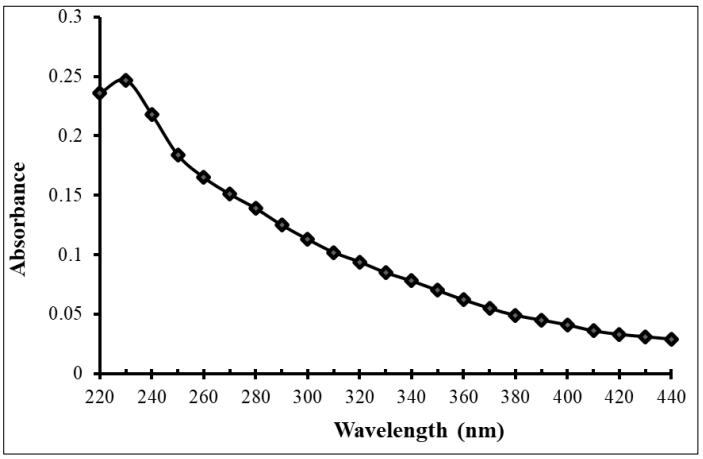

Figure 8 Absorption Spectrum of CLHM-7 Ikot Utin

Figures 7 and 8 show UV absorption spectra of HMS- Ikot Utin before and after chlorination

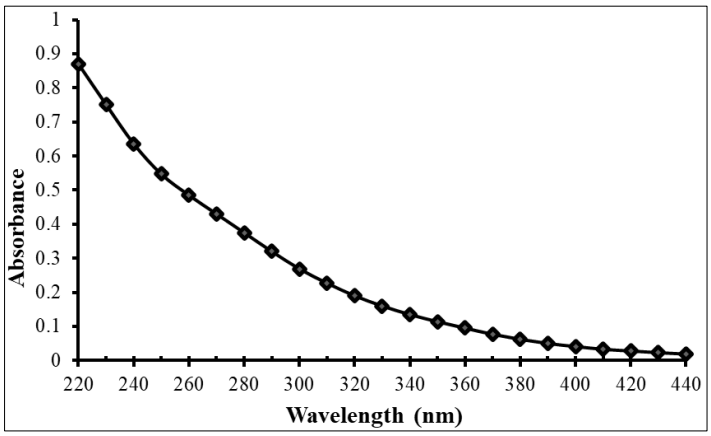

Figure 9 Absorption Spectrum of FHM-1

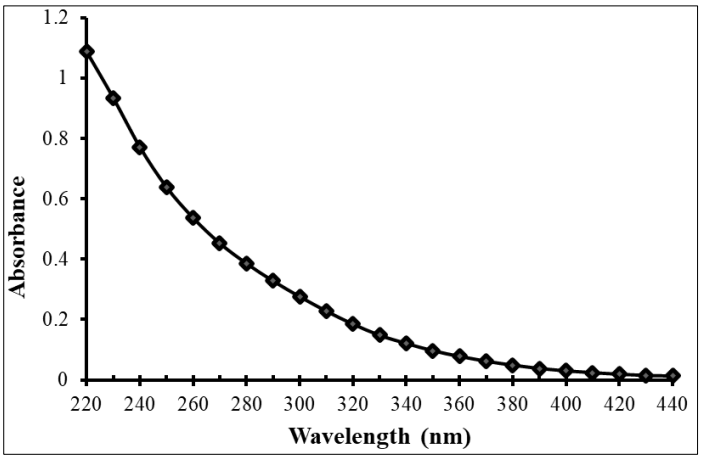

Figure 10 Absorption Spectrum of CFHM-1

Figures 9 and 10 show UV absorption spectra of FHM- 1 before and after chlorination.

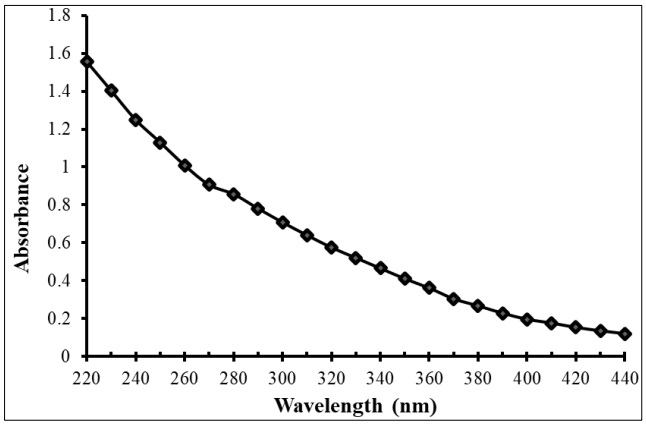

Figure 11 Absorption Spectrum of FHM-2

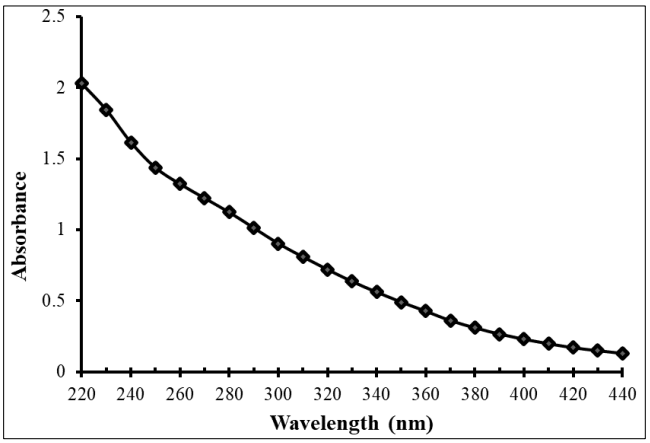

Figure 12 Absorption Spectrum of CFHM-2

Figures 11 and 12 show UV absorption spectra of FHM-2 before and after chlorination

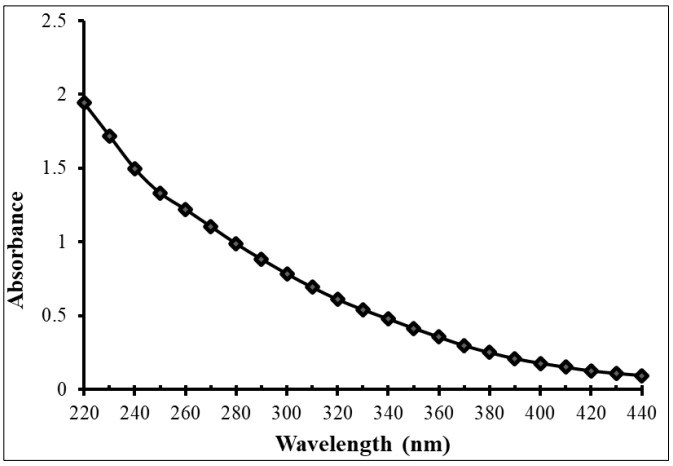

Figure 13 Absorption Spectrum of FHM-3

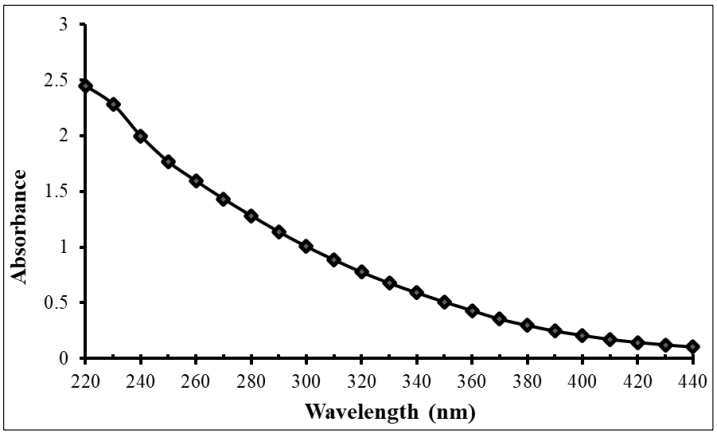

Figure 14 Absorption Spectrum of CFHM-3

Figures 13 and 14 show UV absorption spectra of FHM-3 before and after chlorination

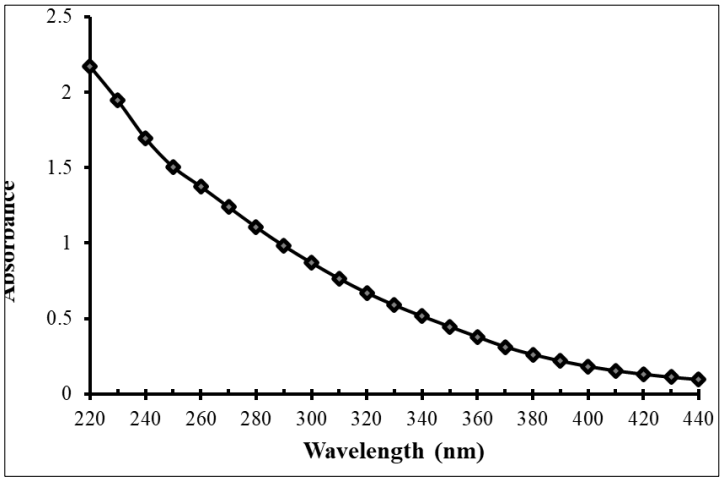

Figure 15 Absorption Spectrum of FHM-4

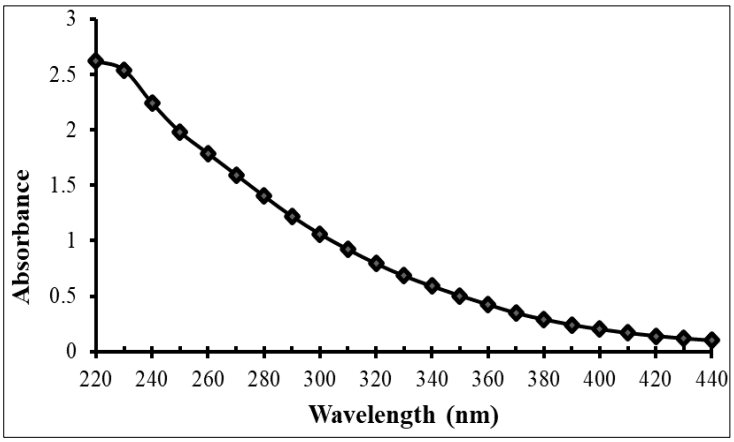

Figure 16 Absorption Spectrum of CFHM-4

UV absorption spectra of FHM-4 before and after chlorination are shown on figures 15 and 16 respectively.

4. Discussion

There is no one single chemical known as humic acid, since the chemical structure has never been completely defined [5]. Humic materials are composed of complicated organic mixtures which are linked together in a random manner, resulting in extraordinarily complex materials. It has been suggested that no two molecules of humus are exactly the same. The special properties of humic materials result from this extreme heterogeneity and their high chemical reactivity. Humic materials have an abundance of carboxyl groups and weakly acidic phenolic groups, which contribute to their complexation and ion-exchange properties.

Because of the comparatively high biocidal effectiveness and relatively simple application, chlorine has become the principal agent for sterilizing potable waters, disinfecting effluents from sewage treatment plants, among others. The use of chlorine in water treatment has been reported to result in the formation of several halogenated substances, and is undoubtedly the primary causative factor of the production of genotoxins in potable water [17]. Its side effects were also questioned [18]. It has been discovered from this present work, that interaction of chlorinated aquatic humic materials from Nigeria, the US and British sources with the liver and kidney *in vitro* resulted in the depletion of GSH concentration of the two tissues. The reduction was found not to be dependent on the source of the aquatic pollutants, but dependent on the structural differences of the pollutants.

Previous work established that the concentration of GSH has a major effect on its antioxidant function [14]. This present study eventually quantified the concentration of GSH in pig liver and kidney. The findings revealed the concentration of GSH in pig liver and kidney to be 0.874 ± 0.003 µmol/g and 0.545 ± 0.002 µmol/g respectively, which is within the concentration range corresponding to normal levels of GSH in mammalian tissues (e.g. 0.5-10 µmol/g [19]. The results showed the concentration of GSH to be higher in the liver than the kidney. This explains the high demand of the antioxidant in the liver for detoxification process, while the kidney is often used for clearance.

It has also been found from the present work that tissues exposed to CAHMs showed significant decrease in the concentration of GSH. This finding is in agreement with the previous work of Günfer T *et.al.,* [20] who reported changes in the levels of MDA and GSH in mice serum, liver and spleen after aluminium administration. The present discovery suggests that the level of GSH was reduced in the two tissues due to conjugation with the aquatic pollutants. This also is in harmony with the reports of [8][9]. GSH depletion was found to be more on tissues exposed to CFHMs than those with CLHMs. This could be due to the fact that the purified CFHMs appeared to be more toxic and concentrated (due to purification process) than the unpurified CLHMs.

5. Conclusion

This study showed the concentration of GSH to be higher in pig liver than the kidney. It further revealed that interaction with aquatic pollutants resulted in depletion of the antioxidant levels in the tissues investigated. Thus re-emphasized the need for amino acids used for cellular synthesis of GSH to be supplemented regularly by consuming important dietary sources of this antioxidant such as fresh fruits and vegetables, fish and meat along with normal meals.

Compliance with ethical standards

Acknowledgments

We acknowledge and appreciate the technical assistance rendered by the laboratory technologist Mr. Aniekan Akpan.

Disclosure of conflict of interest

The authors have no conflict of interest among them.

Statement of ethical approval

Handling of the animals/tissues was approved by the Animal Ethical Committee of the University of Uyo, Uyo, Nigeria. The work was done following the guidelines of the Ethical Committee.

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