

RELATIVE TOXICITY OF CIGARETTE BUTTS LEACHATE AND USEFULNESS OF ANTIOXIDANT BIOMARKER ACTIVITY IN NILE TILAPIA *Oreochromis niloticus* (TREWAVAS, 1983)

***OSUALA, F.I.,¹ ABIODUN, O.A.,² IGWO-EZIKPE, M.N.³ KEMABONTA, K.A.¹ AND OTITOLAJU, A.A.¹**

¹Department of Zoology, Faculty of Science, University of Lagos, P.M.B. 101017, Lagos, Nigeria

² Biological Oceanography Department, Nigerian Institute for Oceanography and Marine Research, Lagos State, Nigeria

³Department of Biochemistry, Faculty of Basic Medical sciences, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria

Abstract

*Relative acute toxicity of cigarette butts leachate, antioxidant biomarkers; superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) activity and lipid peroxidation (LPO), an index of malondialdehyde (MDA) were evaluated in Nile Tilapia (*Oreochromis niloticus*) exposed to two selected commonly consumed brand of cigarettes butts in smoked and unsmoked conditions (SCB 1/ 2 and UCB 1/2) respectively. Under laboratory condition acute toxicity was conducted over a period of 96hrs. Antioxidant activity and lipid peroxidation were based on the derived 96h LC₅₀ value over a period of 28days. On the basis of 96hrsLC₅₀ value, SCB 1 was found to be the most toxic (1.346 cigarette butt/l) followed by SCB 2 (2.271cigarette butt/l), UCB 2 (7.313cigarette butt/l) and UCB 1 (5.559cigarette butt/l) against *O. niloticus*. The results of SOD and CAT activity under the sublethal concentration (1/10th and 1/100th of 96hrs LC₅₀) of cigarette butt leachate showed significantly (P<0.05) decreased activity in liver and gills compared to the control group whereas GSH in the liver and gill was induced significantly (P < 0.05) within 7-14days compared to 21-28days. MDA increased significantly (P< 0.05) in the liver and gill compared to control group. These findings indicate high oxidant activity on the fish, explaining the enormity of the impact of cigarette butt leachates in the environment and the significance of using a set of integrated biomarker in evaluating oxidative stress in aquatic ecosystem.*

Key Words: *Cigarette Butt Leachate, Acute toxicity, Biomarkers, Oxidative stress*

Introduction

The global rise in cigarette consumption is a pressing concern as an estimated 5.6 trillion cigarettes are consumed globally every year and nine

trillion cigarettes are projected for 2025 (Mackay *et al.*, 2006). The impact of cigarette butt waste against organisms is related to their chemical composition, that is over 4000 different chemicals (Slaughter

et al., 2011), their persistence and toxic effects (Lee, 2012). Most of these chemicals are toxic and often leach into aquatic ecosystems, threatening water supply sources and aquatic animals (Novotny et al., 2011) such as water fleas (Register, 2000) and mosquito larvae (Dieng, 2011).

Toxicological data has shown that discarded smoked cigarette butts contain nicotine and heavy metals; arsenic (As), cadmium (Cd), lead (Pb) capable of leaching into surrounding water and eliciting harmful effect on aquatic life. Nicotine is lethal to species of fish (Micevska et al., 2006; Warne et al., 2002), crustaceans, zooplankton and other aquatic organisms (Novotny et al., 2009). Additionally, cigarette butts present ingestion, choking and poisoning hazard to wildlife when erroneously consumed as food (Slaughter et al., 2011; Novotny et al., 2009). Leachates from smoked cigarette tobacco, smoked cigarette filters and unsmoked cigarette filters were acutely toxic to the freshwater cladoceran, *Daphnia magna* between 0.125 and 0.25, 1 and 2, and greater than 16 cigarette butts/L (48-hr LC₅₀), respectively (Register 2000). Slaughter et al. (2011) used the USEPA standard acute fish bioassay technique, and found out that the LC₅₀ for leachate from smoked cigarette butts with remnant tobacco intact was approximately 1.1 cigarette butts/L for both the marine Pacific top smelt (*Atherinops affinis*) and the freshwater fathead minnow (*Pimephales promelas*). Leachate from smoked cigarette filters without tobacco remnants was less toxic than that with tobacco remnants with LC₅₀ values of 4.1 and 5.5 cigarette butts/L respectively for both fish species. Despite the various

researches on the toxicity of cigarette butt leachates to various organisms, it is difficult to assess its risk in intricate mixtures of pollutants especially in aquatic ecosystems in diverse manners (Mari et al., 2009). Thus biomarkers, representing toxicant-induced changes in biological systems, can serve as links between an environmental contamination and its effects, providing unique information on the ecosystem health (Lopez-Lopez et al., 2011). Antioxidant defense systems provide biochemical biomarkers that could be used in environmental monitoring systems (Oruc, 2004). The antioxidant enzymes tend to differ with respect to various types of chemical compounds, therefore the activity of an individual antioxidant enzyme cannot serve as general makers of oxidative damage. Juveniles of *Oreochromis niloticus* (Tilapia), was the choice of the test animal for this study based on its geographic distribution, its availability round the year, short generation time, ability to breeding captivity, sensitivity to toxicants (ELFAC, 1985), high protein content as food, palatability and history of use from inland fisheries (Nelson, 2006). This was the reason it is used to assess the potential ecological risks of cigarette butts (smoked and unsmoked) leachates against aquatic organisms in Nigeria. This study therefore, investigated the relative acute toxicity and biochemical responses in *O. niloticus* utilizing series of antioxidant defense systems as biomarkers of oxidative stress. This will assist in understanding the environmental burden of cigarette butt wastes so that regulatory policies and approaches to disposal of cigarette butt waste may be better justified and designed.

Materials and Methods

Test Animal

Juveniles of *O. niloticus* (mean weight: 6.93cm ± 0.01, mean total length: 8.57 cm ± 0.02) were used in this study. The fishes were purchased from Aquaculture Department of Nigerian Institute for Oceanography and Marine Research (NIOMR) in Badore, Lagos state. The fishes were transported to the ecotoxicology laboratory located in the University of Lagos in oxygenated polythene bags.

Acclimatization

In the laboratory, the fishes were kept in glass holding tanks (50 x 30 x 30cm) containing dechlorinated tap water, acclimatized for 7 days and fed twice daily with feed pellets (Coppens) at 5% body weight. The water was continuously aerated with 220 V air pumps and changed once in 2 days to avoid metabolic accumulation. Stocking and

experimentation was at temperature (27± 3°C) and relative humidity (79±2%) in accordance with guidelines for bioassay techniques (American Public Health Association, 1985).

Test Media

Two different cigarette brand leachate from smoked cigarette butts (SCB 1 and 2) and unsmoked (UCB 1 and 2) with 1cm of remnant tobacco left intact with the filter was used as the test media. The cigarette was purchased from Sabo market, Lagos, Nigeria. The choice was based on the most commonly consumed cigarette in Nigeria.

Preparation of Test Media

Stock solution of cigarettes butt leachate were prepared by submerging smoked and unsmoked cigarette butts in 1000ml of dechlorinated tap water for 24hours. This was serially diluted to obtain the various concentrations for acute toxicity test (Table 1).

Table 1: Cigarette Butt Leachate Concentrations against *O. niloticus*

Cigarette Butt	Concentrations (Cigarette butts/L)						
SCB 1	1.00	1.50	2.00	3.00	3.50	5.00	Control
SCB 2	1.00	2.00	3.00	4.00	5.00	6.00	Control
UCB 1	2.50	4.00	8.00	10.00	12.50	15.00	Control
UCB 2	3.00	5.00	7.50	10.00	12.50	15.00	Control

Bioassay Containers

Bioassays were carried out in glass tanks (22 x 15 x 18 cm) to minimize adsorption of toxicants, risk of corrosion and chemical reactions.

Application of Toxicant to Test Media

Dechlorinated tap water (2000 ml) was measured into bioassay containers containing a predetermined volume of selected brands of cigarette butt leachate (Table 1).

Acute Toxicity Test

Ten fishes of similar sizes in three replicates and untreated control were

introduced randomly into varying concentrations of test media in bioassay containers.

Sub-Lethal (Chronic) Toxicity

Sub-lethal concentrations were derived from the acute toxicity 96hours LC₅₀ values (1/10th and 1/100th) of the cigarette butt leachate for both smoked and unsmoked cigarette prepared from stock solution.

Four active juvenile of *O. niloticus* of similar sizes in two replicate were exposed to the sublethal concentrations for 28days while test media were changed every 4

days to avoid accumulation of metabolic waste. Fishes were feed once daily.

- Smoked (ml/l):1/10th1/100th of 96hours LC₅₀

SCB10.1340.01346

SCB 20.2271

0.02271

- Unsmoked

UCB 10.55590.0559

UCB 20.73130.07313

Biochemical Samples Collection

The liver and gills of sacrificed fishes were harvested on days 7, 14, 21 and 28. These organs were kept in universal bottles in an ice container to maintain the right temperature and transferred to biochemistry laboratory for analysis.

Biochemical Analysis

Estimation of Protein concentration was determined using Biuret method (Gornall, 1949), LPO an index of MDA levels was determined applying a modified method of Luotola and Luotola (1985), GSH determination as described by Sedlak and Lindsay (1968), SOD as described by Sun and Zigma (1978) and CAT by the method of Beers and Sizer as described by Usoh *et al.* (2005).

Assessment of Quantal Response

O. niloticus were assumed dead if there was no movement of appendages, opercula, mouth or tail when displaced with forceps or glass rod.

Statistical analysis

Toxicological dose-response data (mortality) were analyzed using Probit by Finney (1971) based on a computer program written by Ge Le Pattoourel (Imperial College, London) as adopted by Don-Pedro (1989).The biochemical data were analyzed using Duncan multiple range test following One-way Analysis of Variance (ANOVA) using SPSS 10.0 (SPSS Inc., Chicago, USA). Differences at $P < 0.05$ were considered significant.

Results

Acute Toxicity

On the basis of 96hrLC₅₀ value, SCB 1 (1.346cigarette butt /L) was more toxic than SCB 2 (2.271 cigarette butt /L) against *O. niloticus*. Similarly, UCB 1 was more toxic (5.559 cigarette butt/l) than UCB 2 (7.313cigarette butt /l). The computed toxicity factor (96hrLC₅₀) revealed that SCB 1 was about 2.35x, 3.14x, 4.74x and 5.3x more toxic than SCB 2, UCB 1 and UCB 2 respectively (Table 2).

Table 2: Relative Acute Toxicity of Cigarette Butt Leachate

Treatment	Time (hr)	LC ₅₀ (95% CL)mg/L	LC ₉₅ (95% CL)mg/L	Slope ± S.E	Probit line equation	DF	TF
SCB 1	24	8.167(3.938-0.662)	49.63(9.858-1.106)	0.575+2.09	Y=1.57+1.67 * X	3	1
SCB1	48	8.531(4.760-1.987)	287.24(5.098-1.234)	0.775+0.694	Y=-1.00+1.1429 * X	3	1
SCB1	72	2.68(2.987-1.236)	68.381(19.09-4.093)	0.869+0.646	Y= -0.5+1.429 *X	3	1
SCB1	96	1.346(1.879-0.540)	7.25(11.48-4.954)	0.977±0.283	Y=1.025±3.122X	3	1
SCB 2	24	8.35(7.987-5.176)	13.48(9.765-2.918)	0.236±6.675	Y= 2.91±4.800X	3	1.02
SCB 2	48	20.01(2.398-0358)	668(23.33-13.98)	0.796+0.404	Y= 1.4 ±1.25 *X	3	2.35
SCB 2	72	5.313(337-3.210)	82.66(1.24E1017.16)	0.910+0.352	Y=-1.00±1.5625 *X	3	1.98
SCB 2	96	2.271(3.123-1.338)	12.01(70.12-6.842)	0.911±0.334	Y=2.425±3.596X	3	1.69
UCB1	24	25.67(4.44-1.168)	164.24(1.55XE10)	0.926+0.901	Y= -2.4+1.6667 X	3	3.14
UCB1	48	18.472(2.326-1.70)	132.433(5.655-1.600)	0.954+0.746	Y=-1.8+1.3333 * X	3	2.17
UCB1	72	12.699(1.723-0.733)	122.85(5.552-1.609)	0.922+0.623	Y= -1.68+1.4 * X	3	4.74
UCB1	96	5.559(8.665-4.346)	28.319(2.638-1.240)	0.981±0.520	Y= 2.778+3.339X	3	4.79
UCB 2	24	17.718(2.251-1.176)	36(4.372-1.333)	0.798±2.43	Y= -4.00+3.00 * X	3	2.18
UCB 2	48	15.004(1.554-1.054)	51.054(2.977-1.414)	0.884±1.002	Y=-2.8±2.3333 * X	3	1.76
UCB 2	72	9.09(1.097-0.848)	31.98(2.118-1.290)	0.951±0.771	Y= -2.75+3.125* X	3	3.39
UCB 2	96	7.313(9.311-5.206)	25.38(81.39-16.38)	0.951±0.77	Y= -2.75+3.125X	3	5.33

D.F =Degree of Freedom, C.L =95% Confidence Limit
 Toxicity Factor(T.F) = $\frac{24/48/72/96\text{hrs LC}_{50} \text{ value of other cigarette butt}}{24/48/72/96\text{hrsLC}_{50} \text{ of the most toxic}}$ S.E=Standard Error,

Biochemical studies

Generally, the results of LPO measured through MDA in the organs of *O. niloticus* showed that there was significant (P < 0.05) difference between the levels of MDA observed in the control compared to all treated groups in the liver and gills. Exposure of *O. niloticus* to UCB 1 showed that MDA measured in the liver increased from 7.88 to 8.09 μmol/mg whereas in UCB 2, it range from 8.31 μmol/mg to 12.05 μmol/mg in the 1/10th and 100th sublethal concentrations respectively (figs. 1 and 2), whereas SCB1 showed increased level of MDA from 9.08 to 11.68 μmol/mg while in SCB 2, it ranged from 8.86 to

9.37 μmol/mg which was significantly (P < 0.05) different from that of control 5.14 μmol/mg (figs. 3 and 4).

MDA induction in the gill ranged from 8.94 - 13.58 μmol/mg (UCB 1) and 6.43- 10.26 μmol/mg (UCB 2) while MDA level in SCB 1 varied from 9.65 - 29.54 μmol/mg whereas SCB 2 range from 13.69- 16.42 μmol/mg in the 1/10th and 1/100th respectively. Additionally the MDA level increased significantly (P< 0.05) in the gill compared to the liver in both SCB 1 and 2 (Fig 3 and 4) while in the UCB 1 and 2; the MDA level showed a slight increase in the liver compared to the gill (1 and 2).

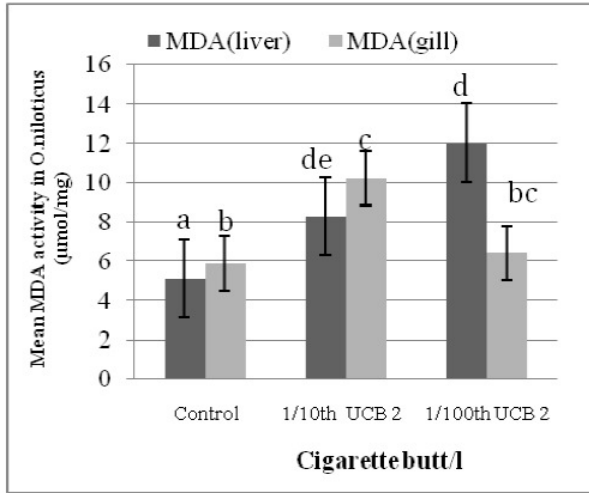


Fig. 1: MDA in liver and gill of *O. niloticus* treated with cigarette butt leachates. Dissimilar letters are significantly ($P < 0.05$) different (N = 4)

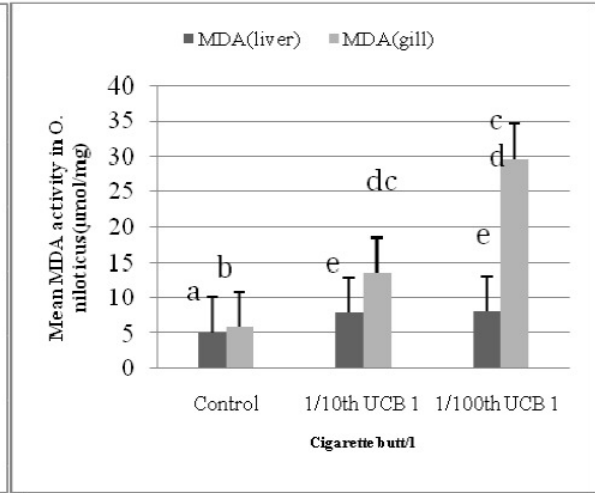


Fig. 2: MDA in liver and gill of *O. niloticus* treated with cigarette butt leachates. Dissimilar letters are significantly ($P < 0.05$) different (N = 4)

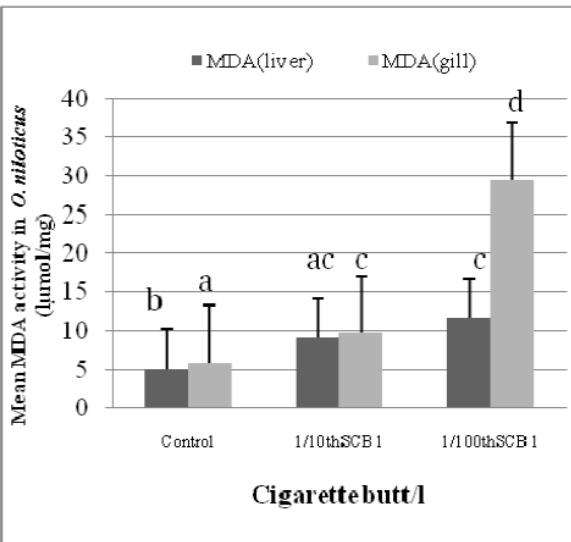


Fig 3: MDA level in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N=4)

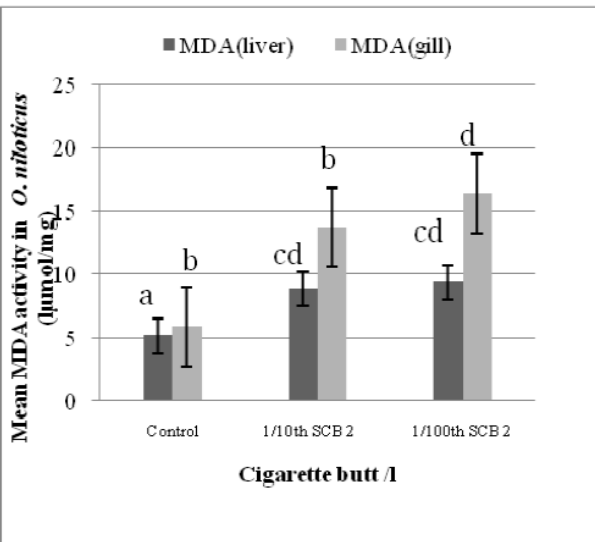


Fig.4: MDA level in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N=4)

Results of GSH levels in liver and gill showed significant ($P < 0.05$) differences between control and all treated groups. GSH in the liver was 40.38 - 45.48 µmol/mg and 41.88 - 56.78 µmol/mg (UCB 1 and UCB 2) respectively whereas it was 54.61 - 55.07 µmol/mg and 46.14 -

46.72 µmol/mg in SCB 1 and SCB 2 (figs. 5 and 6).

In the gill, GSH level ranged from 45.06 - 45.48 µmol/mg in 1/100th and 1/10th of UCB 1 respectively while in UCB 1 was 41.84 to 43.94 µmol/mg relative to control of 26.82 µmol/mg. GSH level varied from

45.48 to 46.60 $\mu\text{mol}/\text{mg}$ in SCB 1 whereas in SCB 2 it ranged from 48.34 to 51.28 $\mu\text{mol}/\text{mg}$. Additionally the GSH level increased significantly ($P < 0.05$) in the gill

compared to the liver in both SCB(1 and 2) exposure while in the UCB 1 and 2, it showed a slight increase in the liver compared to the gills.

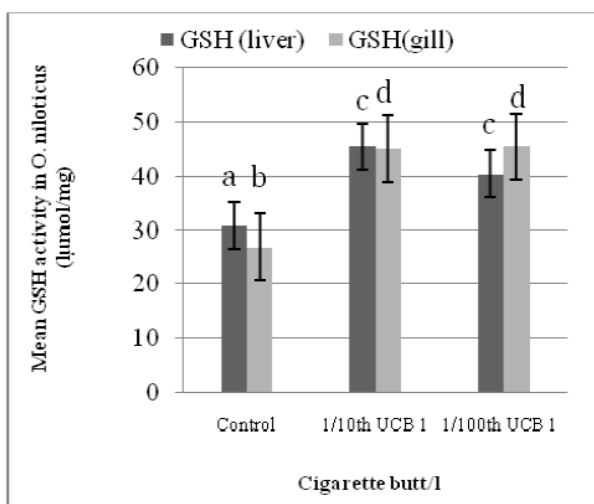


Fig. 5: GSH in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

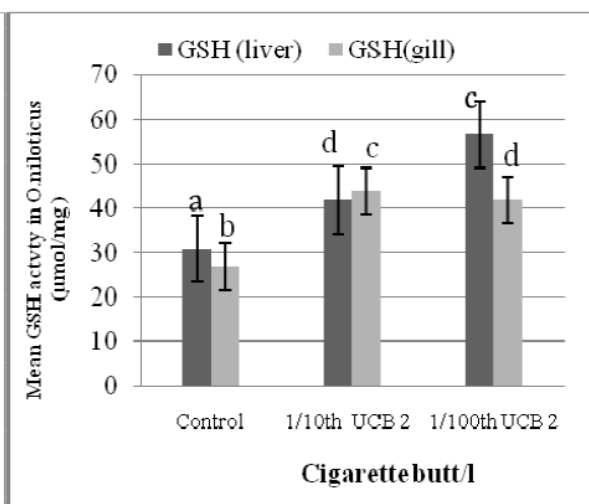


Fig. 6: GSH in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

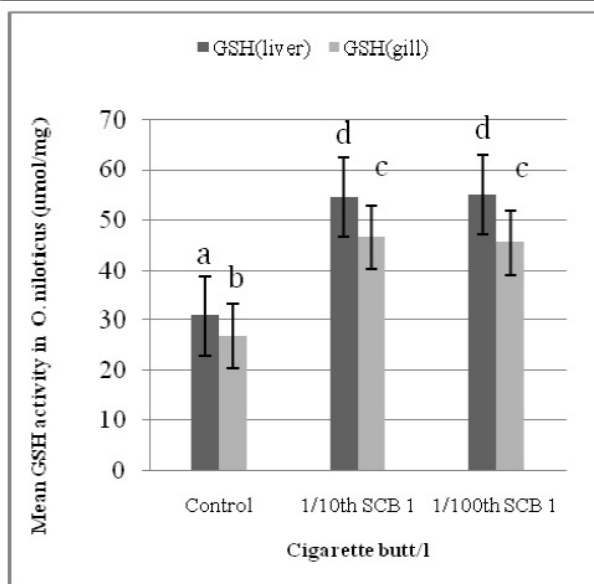


Fig. 7: GSH in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

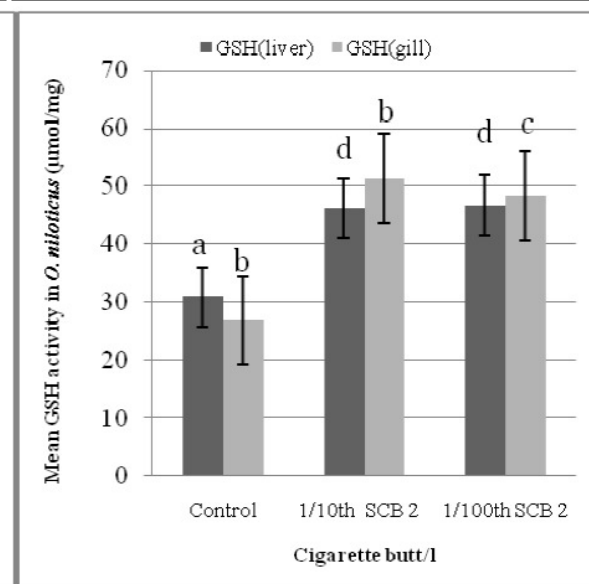


Fig. 8: GSH in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

SOD activity in the liver were 134.75 to 154.00 $\mu\text{mol}/\text{mg}/\text{proteins}$ and 104.00 to

108.00 $\mu\text{mol}/\text{mg}/\text{proteins}$ (UCB 1 SCB 1) whereas it ranged from 160.75 to 161.75

μmol/mg/proteins and 124.00 to 132.00 μmol/mg/proteins in (UCB 2 and SCB 2) respectively which were significantly (P < 0.05) different to that in control 187.00 μmol/mg/proteins (figs. 9 to 12).

In the gill SOD levels were 114.96 to 147.57 μmol/mg/proteins and 95.72 – 125.92 μmol/mg/proteins (UCB 2 and SCB 2) respectively whereas it was 83.92

– 103.73 μmol/mg/proteins and 68.71 – 83.92 μmol/mg/proteins (UCB 1 and SCB 1) that were significantly (P < 0.05) to that in control (177.25 μmol/mg/proteins). Comparison of SOD level in the gill and liver relative to the control showed they were significantly (P < 0.05) different (figs. 9 - 12) from each other.

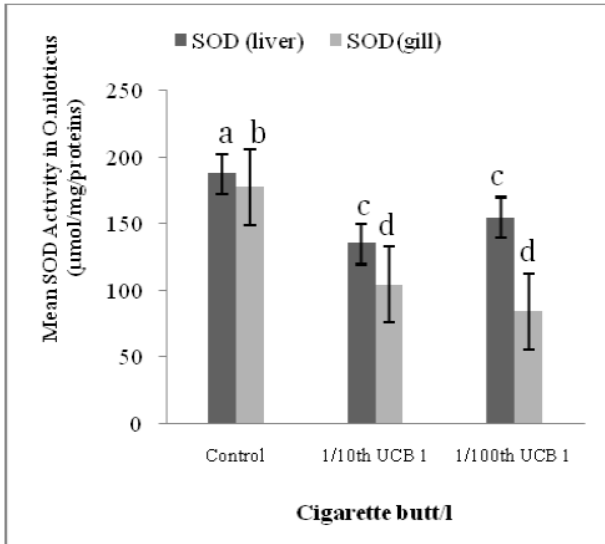


Fig. 9: SOD in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly (P < 0.05) different (N = 4)

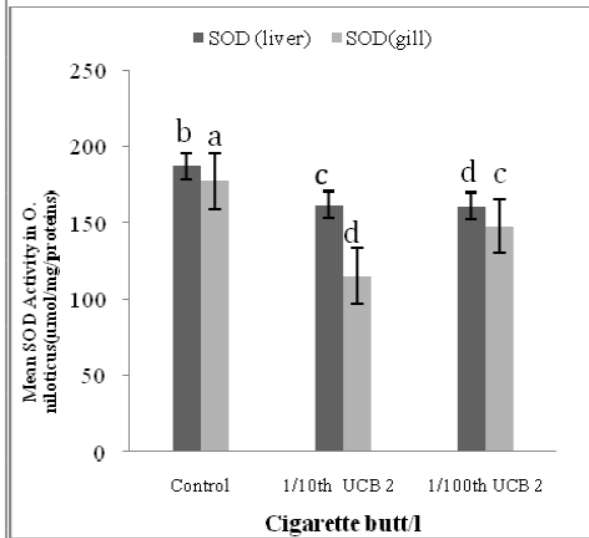


Fig.10: SOD in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly (P < 0.05) different (N = 4)

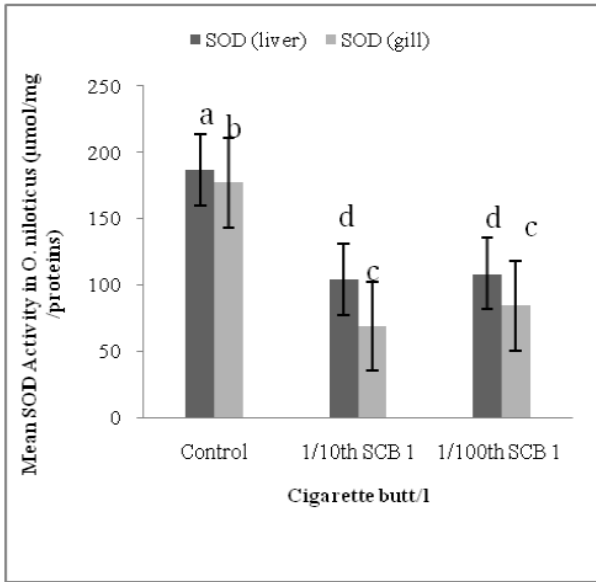


Fig. 11: SOD in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

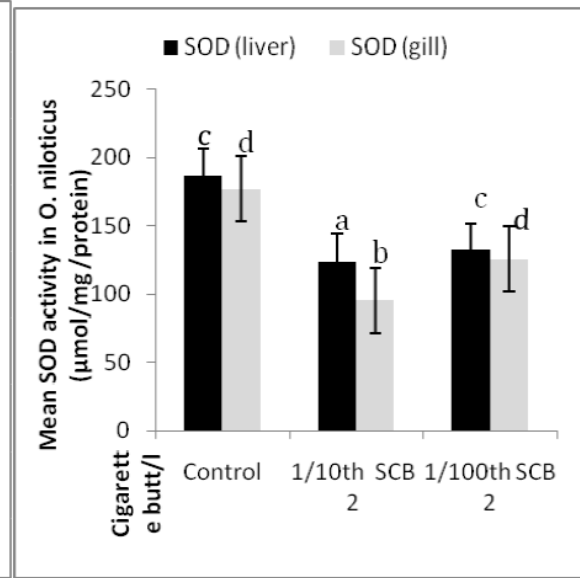


Fig. 12: SOD in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

Results of CAT activity in the liver were 149.50 – 261.50 $\mu\text{mol/mg/proteins}$ and 118.50 – 227.50 $\mu\text{mol/mg/proteins}$ (UCB 1 and SCB 1) while (UCB 2 and SCB 2) range from 387.75 – 476.25 $\mu\text{mol/mg/proteins}$ and 176.75 – 375.25 $\mu\text{mol/mg/proteins}$ respectively which were also significantly ($P < 0.05$) to that in control 746.75 $\mu\text{mol/mg/proteins}$ (figs. 13-16).

In the gill CAT levels ranged from 289.74 – 370.26 $\mu\text{mol/mg}$ and 190.18 –

370.26 $\mu\text{mol/mg/proteins}$ (UCB 1 and SCB 1) whereas (UCB 2 and SCB 2) CAT level ranges between 317.91- 420.61 $\mu\text{mol/mg}$ and 272.18 – 305.83 $\mu\text{mol/mg}$ respectively which were significantly ($P < 0.05$) different to that in control 746.75 $\mu\text{mol/mg/proteins}$ (figs. 13-16). Comparison of CAT induction in the gill and liver relative to the control showed they were significantly ($P < 0.05$) different from each other.

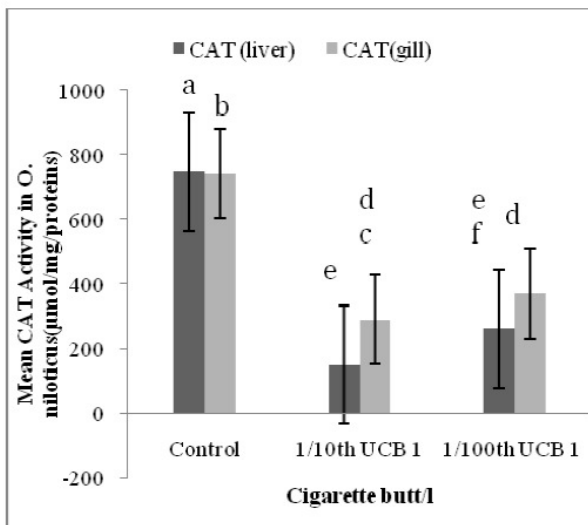


Fig. 13: CAT in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

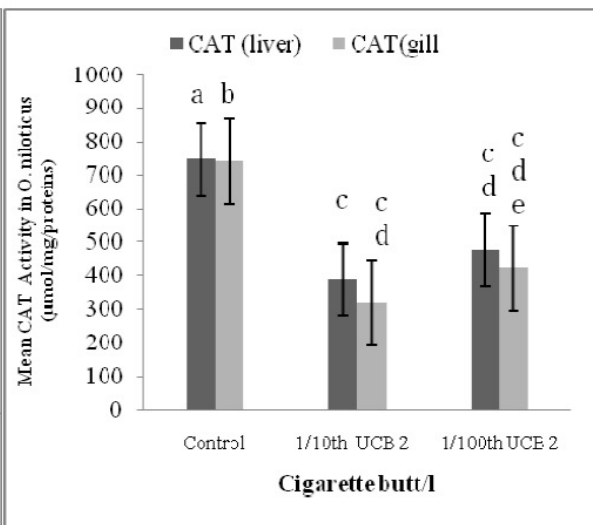


Fig. 14: CAT in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

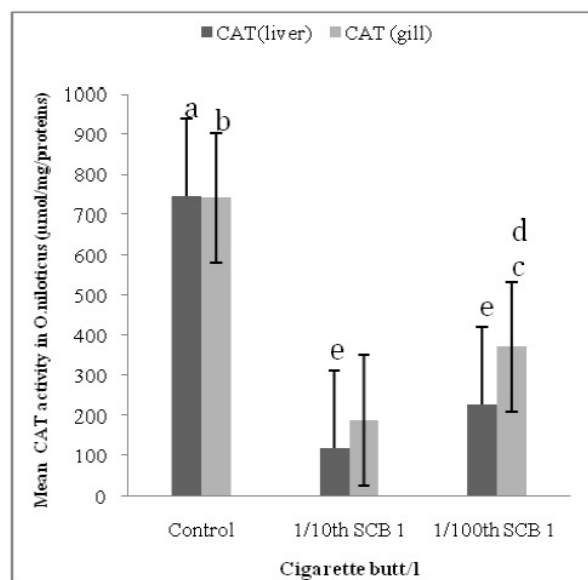


Fig. 15: CAT in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

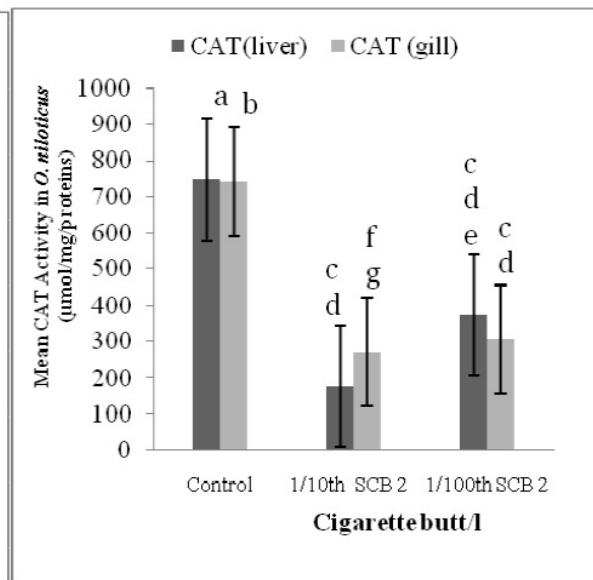


Fig. 16: CAT in liver and gill of *O. niloticus* treated with cigarette butt leachate. Dissimilar letter are significantly ($P < 0.05$) different (N = 4)

Discussion

The study evaluated the acute and sublethal toxicity of smoked and unsmoked cigarette butt leachates using LPO and antioxidant defense systems as biomarkers. As established SCB 1 which

was the most toxic relative to SCB 2 was in agreement with the findings of Micevska *et al.* (2006). The authors showed that smoked cigarette butt leachate was acutely toxic to the daphnid, *Ceriodaphnia dubia* at concentrations

between 8.9 and 25.9cigarette butts/L when remnant tobacco was left intact. Given that the mean weight of a single smoked cigarette butt used in this study was approximately 310 mg, it could be calculated that smoked cigarette butt leachate which was found to be acutely toxic to *O. niloticus* was between 0.004 and 0.007cigarette butts/L 48-hr EC₅₀ (immobilization). In comparison, the current study found smoked cigarette butt leachate to be toxic to *O. niloticus* at the LC₅₀ (96-hr) at 1.346 cigarette butts/l in SCB 1 compared to SCB 2 with LC₅₀ of 2.271 cigarette butts/l.

A probable reason for the acute toxicity of these cigarette butt leachates could have been as a result of retained pesticides from agricultural field. A study performed by Dane *et al.* (2006) confirmed flumetralin, pendimethalin, and trifluralin pesticides were previously detected in both mainstream and side stream cigarette butt. Pesticides have been shown to be more toxic to water fleas (*D. magna* and *C. dubia*) than to fish (*P. promelas* and *A. affinis*). This finding supports the possibility that pesticides could have been retained in the cigarette butt. Given that many cigarette additives are carcinogenic to humans, it may follow that they probably will also be toxic to aquatic organisms. Chemical additives are a significant component of cigarettes comprised of approximately 10% of the cigarette by weight and could possibly serve to impart toxicity to aquatic organisms (Iskander, 1985).

The toxicity of UCB 1 and 2 to fish concur with the findings of Warne *et al.* (2002) and Register (2000). Consequently, leachate from unsmoked cigarette butt was least toxic compared to leachate from smoked cigarette butt. The results showed

that the chemicals solely in the smoked butt still exert considerable toxicity to fishes. Some possible explanations for this trend of increased toxicity with smoked cigarettes are that smoking may create new, more toxic chemicals. For example, PAHs, furans, and benzene are all toxic products of combustion and have all been found to occur in cigarette smoke (Agency for Toxic Substances and Disease Registry, 1995). Smoking changes the solubility of compounds in cigarette butts, making them more bioavailable (Hoffmann and Hoffmann, 1997). PAHs found in cigarette smoked are capable of bioaccumulating in the tissues of fish (Agency for Toxic Substances and Disease Registry, 1995; Hoffmann and Hoffmann, 1997). The act of smoking cigarettes may also increase the concentration of toxicants in the cigarette butt as more chemicals are pulled through and retained by the filter as smoking continues.

The increased level of MDA in the gills and liver was probably indicative of the potential to cause oxidative injury (Vlahogianni, 2007). Gills are the first organs in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals due to their large surface area that facilitates greater toxicant interaction and absorption with a detoxification system that is not as strong as that of liver (Yildirim, 2011). This finding further confirms the fact that cigarette butts leachate could cause deleterious effects.

GSH as antioxidants protects the system from oxidative attacks by reactive oxygen species (ROS) because they act as reducing agent and free-radical trapper. Therefore, decreased GSH level in the liver and gills possibly demonstrated the inefficiency of these organs in neutralizing

the impact of ROS, resulting in increased LPO that could have resulted in oxidative stress. These results are similar to those of Mather-Mihaich and DiGiulio (1986). GSH depletion seems to reflect an aggravation status probably due to reduced cell protection ability (Yin, 2007). Zhang *et al.* (2004) noted that severe oxidative stress could suppress GSH levels due to the impairment of adaptive mechanisms. Therefore, decreased GSH level might have indicated inability to confer protection. Decreased activities of the SOD and CAT enzymes observed on the 21st-28th day may be related to pollutants that increase ROS production resulting in oxidative stress. Usually a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants that are in agreement with this current study (Dimitrova *et al.*, 1994). The biological importance of CAT are more evident from various studies (Mari *et al.*, 2009) due to the fact that H₂O₂ is the main cellular precursor of the hydroxyl radical which is a highly reactive and toxic form of ROS (Lopez-Lopez *et al.*, 2011; Mather-Mihaich and Di-Giulio, 1986). The elevated CAT activity in 1/10th and 1/100th of SCB and UCB in 7th day indicated that it could be induced in order to resist the pollutants toxicity. However, reduced CAT activity from day 14th to 28th probably indicated that enzymatic biomarkers of oxidative stress could be sensitive indicator of aquatic pollution. This could be related to the alterations in antioxidant enzymes activities in *O. niloticus* exposed to smoked and unsmoked cigarette butts leachate. Under acute oxidative stress, the toxic effects of pollutants may overwhelm the antioxidant defenses systems (Bebiano *et al.*, 2004). This showed that the multitude of biomarkers used in this

study can serve as a good battery indicator of the impact of cigarette butts leachate on the fish species in an integrated manner.

Conclusion

Cigarette butt is one of the most common forms of litter in the world, and this study has established the fact that cigarette butts leachate are toxic to *O. niloticus* thus strict environmental protection should be ensured. Additionally, the biomarkers are good potential tools that can serve to improve the assessment of biologically significant exposures to cigarette butt leachate and enhance monitoring of pollutants on the health and survival of exposed populations.

Recommendation

O. niloticus exhibited sensitivity to cigarette butt leachate thus further research should be geared towards the actual impact of cigarette butt leachate on other aquatic organisms in freshwater and marine environment, their bioaccumulative potential and joint action toxicity.

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