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Pollutant mixtures as stressors of selected enzyme activities of the aquatic snail *Helisoma duryi*

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This study involves an investigation on the effects of a pesticide, metal and a detergent as individual and mixtures on esterase and antioxidant enzyme activity of the freshwater snail *Helisoma duryi*. Adult snails were exposed to sublethal concentrations of copper (5 µg/L), industrial detergent, oxyfoam (15 µg/L), carbaryl (25 µg/L) as well as mixtures of these pollutants for 96 hours. Carboxylesterase and cholinesterase activities were measured using 4-nitrophenyl acetate and acetylthiocholine iodide as substrates respectively. The activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S- transferase were also measured as indices of oxidative stress. Esterase activity was inhibited in snails exposed to carbaryl, copper or detergent. Mixtures of the different chemicals also caused inhibitions of esterase activity when compared to the controls. All the chemicals individually and as mixtures, caused enhanced activities of antioxidant enzymes. When comparing all the antioxidant enzymes analyzed, the highest activity was caused by the triple mixture of the pollutants. The results suggest that aquatic life is at risk to adverse effects of pollutant mixture as reflected by increased alteration of enzyme activity in mixture- exposed snails though the increase was less than sum of effects of individual pollutants. More studies on the effects of a wider range of pollutant mixtures on aquatic organisms are needed however, for the full appreciation of reactive interactions that takes place in complex mixtures which ultimately affect the health of aquatic biota.

Key words: Pollutants, snails, oxidative stress, esterases, detergents.

INTRODUCTION

Human activities carried out in man's quest to increase food security and uplift his living conditions directly and indirectly produce chemical pollutants which severely affect the condition of aquatic ecosystems worldwide (Maceda-Veiga et al., 2010; Belabed and Soltani, 2013). Chemicals from agricultural activities enter aquatic bodies via aerial drifts and runoffs after heavy rains (Wang et al., 2010), while those from domestic and industrial activities mostly enter aquatic bodies via wastewater treatment facilities (Ghoochani et al., 2011; Dan'azumi and Bichi, 2010; Muchuweti et al., 2006). While municipal and industrial companies are expected to pass only treated

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water to aquatic reservoirs, burst pipes, old unmaintained pipes and heavy load on equipment in these facilities sometime result in partially and /or untreated effluent from industries and wastewater treatment plants entering aquatic bodies where they cause deleterious effects on aquatic life (Sankpal and Naikwade, 2012; Nyamangara et al., 2008; Oberholster and Ashton, 2008).

Aquatic bodies being the final sink receives effluent from different sources and these chemical pollutants form chemical mixtures which exert various biochemical effects on the wellbeing of aquatic biota. The chemicals interact additively, antagonistic or synergistically amongst themselves which affect their overall effect on the aquatic biota (DeLorenzo and Serrano, 2003). There is need therefore, to monitor the effects of chemical mixtures on aquatic biota to safeguard the health of aqua-ecosystems.

Aquatic reservoirs rarely contain one chemical pollutant at a time. Being the ultimate sinks, chemicals may come from various sources and this means that the aquatic organisms residing in these waters are constantly being exposed to mixtures of chemicals which undoubtedly affect the wellbeing of these organisms. Literature reports in most countries, Zimbabwe included (Siwela, 2008; Nyamangara et al., 2008), specify effects of known chemical pollutants on aquatic biota, however, very little information is available on the effects of complex mixtures of chemicals on the wellbeing of aquatic organisms. Measurement of residue levels of pollutants in aquatic bodies remain a challenge because the concentrations of the pollutants that enter aquatic ecosystems generally fall below levels that analytical instruments can detect. Most researchers have now adopted biomarkers to detect exposure and effects of environmental pollutants in aquatic environments. Environmental pollutants are generally lipophilic and easily move and accumulate in fatty tissues where they can easily be detected when tissue samples are being analyzed. The use of biological markers at the molecular or cellular level has thus been proposed as a sensitive early warning tool for biological measurement of pollution (Van der Oost et al., 2003)

The effects of a pesticide, metal and detergent as individual as well as mixtures on esterase and antioxidant enzyme activities of the aquatic snail *H. duryi* was investigated in order to assess the potential of these biochemical endpoints as biomarkers of exposure to mixtures of chemical pollutants in aquatic ecosystems. Carbaryl was chosen as a pesticide because of the popular use in the country and wide spectrum of target pests as an insecticide, acaricide and molluscicide (US EPA, 2003). Copper was the metal pollutant of choice as exposure to this heavy metal has increased as the country builds new houses to meet one of its goals of improving the living conditions of all nationals. New houses come with new plumbing pipes that carry potable water and these pipes are usually made of copper. Copper fungicide is also commonly used in agriculture (US EPA, 2006).

Industrial activities continue to increase in Zimbabwe as in many developing countries resulting in increased output of effluent from different industries. Detergents are extensively used in industries as well as in homes and effluent containing detergents can reach aquatic bodies if industrial companies dispose untreated water or if equipments at wastewater treatment plants fail to function properly (Ghoochani et al., 2011; Mahvi et al., 2004; Kowalska et al., 2005). Oxyfoam, a high strength peroxide based detergent foam cleaner was chosen because of its wide range use in cleaning dairy, beverage, water and food processing equipment. Molluscs are frequently used to biomonitor heavy metals and organic pollutants such as pesticides in aquatic ecosystems because they possess essential characteristics required for bioindicator species (Wagner and Boman, 2004). Molluscs are effective accumulators of pollutants (Ravera, 2001) because they filter great volumes of water with soluble and particulate substances, which, after being metabolised, are selectively concentrated in their soft tissue or in the shell. In addition, they are sedentary, easy to culture in the laboratory or to maintain in cages during field studies which makes them ideal bioindicator organisms. Pollution induced cellular or tissue damage and other biochemical responses to xenobiotics in both marine and freshwater species indicate the usefulness of molluscs as sensitive indicators of environmental pollution (Guerlet et al., 2006; Gagnaire et al., 2008; Nawel et al., 2012).

The test organism for the present study, *Helisoma duryi* are common elements of freshwater biota throughout most Sub-Saharan Africa, including Zimbabwe (Brown, 1994). They are widely spread throughout the country's aquatic reservoirs, are easy to breed, mature early and start breeding after about one and half months from the day they hatch and grow quickly passing through multiple generations in a single season (Brown, 1994). Despite having the qualities of ideal bioindicator species, very few studies have exploited these organisms as biomarkers of water pollution.

MATERIALS AND METHODS

Chemicals

All chemicals and biochemicals were purchased from Sigma Aldrich Chemical Company, Germany. Bovine serum albumin was of 98% purity. Carbaryl was a pure standard of ≥ 98% purity. The detergent, oxyfoam was obtained as a kind donation from Zimbabwe Pharmaceuticals (Pvt). Ltd, and was of technical grade. All other laboratory reagents were of analytical (ANALAR) grade.

Snail breeding and exposure

Helisoma duryi snails were bred in cement outdoor aquaria containing tap water and fed on fresh garden lettuce according the method of Naik and Hasler (2002). Snails of approximately 20 ± 5

mm in shell diameter were used in the study. Groups of adult snails (20 per group) were exposed to 5 ppb copper, 15 ppb oxyfoam (a detergent) and 25 ppb carbaryl, 20 ppb metal and detergent mixture, 30 ppb metal and pesticide mixture, 40 ppb detergent and pesticide mixture and 45 ppb metal, pesticide and detergent mixture for 96 h. The exposure studies were done in 1 L volume of municipality water at room temperature.

The concentrations of individual pollutants used in the present study were reported levels of pollutants in the dams adopted from literature (Maredza and Naik, 1998; Siwela et al., 1996; Siwela, 2008; Siwela et al., 2010). Use of levels of pollutants already reported were adopted because these have an environmental significance and provides information on effect of levels of pollutants that are actually encountered by these freshwater organisms in the their natural habitats. The exposures were done in quadruplicates. There were no mortalities recorded during the exposure period. Water, food and pesticides were refreshed every 24 h. After the exposure period the snails were homogenized to prepare the post mitochondrial fractions.

Preparation of snail homogenates

Snails from each group were de-shelled before being pooled and homogenized, in 3 volumes of ice-cold buffer (0.1 M potassium chloride buffer, pH 7.4 containing 1.15% potassium chloride). The homogenates were then centrifuged at 10 000 X*g* for 10 m. The pellets were discarded and the supernatants (PMFs) were kept at -80°C until analyzed.

Assessment of enzymatic activity

Cholinesterase activity

Cholinesterase activity was measured using the substrate acetylthiocholine iodide according to the method described by Ellman et al. (1961) that was modified for a plate reader by Kallander et al. (1996). The reaction mixture contained 50 µL of 0.1 mg/mL PMF, 110 µL of 0.01 M Tris/HCl buffer pH 8.0 and 50 µl of 0.4 mM 5, 5 dithio-bis-(2 nitro benzoic acid) DTNB. The mixture was incubated for 3 m before adding 30 µL of 0.5 mM acetylthiocholine iodide. The rate of production of a complex between thiocholine and DTNB was followed for 3 m at 412 nm.

Carboxylesterase activity

Carboxylesterase activity was measured using 4-nitrophenyl acetate as the substrate, following the method of Mackness et al. (1983). The following reagents were added to a microtitre plate: 50 µL of 0.1 mg/mL PMF, 200 µL of 0.02 M Tris/HCl buffer pH 7.6 and 0.024 M of 4-nitrophenol acetate in ethanol. Rate of production of 4-nitrophenol was followed for 5 minutes (37°C) at 400 nm using a SpectraMax 340pc plate reader.

Superoxide dismutase activity

Superoxide dismutase activity was measured following the method of Yi et al. (1988). Xanthine and xanthine oxidase were used to generate superoxide anion radicals, which react with 2-(4-indophenyl)-3(4-nitro-phenyl)-5-phenyl tetrazolium chloride (NBT) to form a red formazan dye. The reaction mixture contained 0.5 mL sample or standard Cu, Zn-SOD and 2.45 mL SOD Assay Reagent (SODAR). The SODAR contained 40 mL of 0.3 mM xanthine, 20 mL of 0.6 mM ethylenediaminetetraacetic acid (EDTA), 12 mL of 400 mM sodium carbonate, 6 mL of 0.1% w/v bovine serum albumin and 20 mL of 150 µM NBT. The working range for the Cu, Zn-SOD standard curve was 0 – 300 ng/mL. One enzyme unit of superoxide dismutase is defined as the amount, which inhibits the NBT reaction by 50%. Specific activity was defined as units/mg protein.

Catalase activity

Catalase activity was assayed according to the method of Claiborne (1989). The following reagents were added to a 3 mL quartz cuvette: 2950 µL of 19 mM hydrogen peroxide solution in 50 mM potassium phosphate buffer (pH 7.0) and 50 µL of sample to bring a final volume in the cuvette to 3000 µL. The blank had 2950 µL of buffer with no substrate. The rate of decrease in absorption was monitored on a Hewlett Packard Lamda 2 UV/Vis spectrophotometer (240 nm, 25°C).

Glutathione peroxidise activity

Glutathione peroxidase activity was determined in PMFs following the method of Flohe and Gunzler (1984). The following reagents were added in a microtitre plate: 115 µL of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 25 µL of sample, 10 µL of 1 mM sodium azide, 25 µL of glutathione reductase (2.4 units/mL) and 25 µL of 10 mM GSH. The blank contained 25 µL of phosphate buffer in place of sample. The reaction mixture was incubated at 37°C for 10 minutes before addition of 25 µL of 1.5 mM NADPH in 0.1% w/v sodium bicarbonate. Hydroperoxide-independent NADPH consumption was measured at 340 nm. After following the decrease in absorbance for 3 m, 25 µL of 1.5 mM hydrogen peroxide was added and the overall decrease in absorbance of NADPH with time was followed at 340 nm using a SpectraMax 340pc plate reader for 5 m. Enzyme activity was expressed as micromoles GSH oxidized/min/mg protein.

Glutathione S-transferase activity

Glutathione *S*-transferase activity was measured in S-10 fractions according to the method of Habig et al. (1974). The reaction mixture contained the following reagents: 200 µL of 0.1 M sodium phosphate buffer (pH 6.5), 30 µL of sample, 10 µL of 25 mM GSH and 10 µL of 25 mM CDNB. Rate of production of the 1-chloro-2-4-dintrobenzene-glutathione conjugate (CDNB–GSH) formed was followed at 340 nm using a SpectraMax 340pc plate reader Enzyme activity was expressed as millimoles of CDNB–GSH formed/min/mg protein.

Protein determination

Protein concentration was measured in S-10 according to method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

The analysis of enzymatic activities were performed in quadruplicates and the data was reported as mean $\pm$ SD of calculated specific activities of enzymes of each exposure study. The graphical presentations were performed using the GraphPad prism 4 program or Microsoft Excel. The statistical differences between the exposed snail pooled samples and the controls (unexposed snail pooled samples) were tested using the GraphPad Instat 3. The significance of the results was ascertained at $*p<0.05$ and $**p<0.01$.

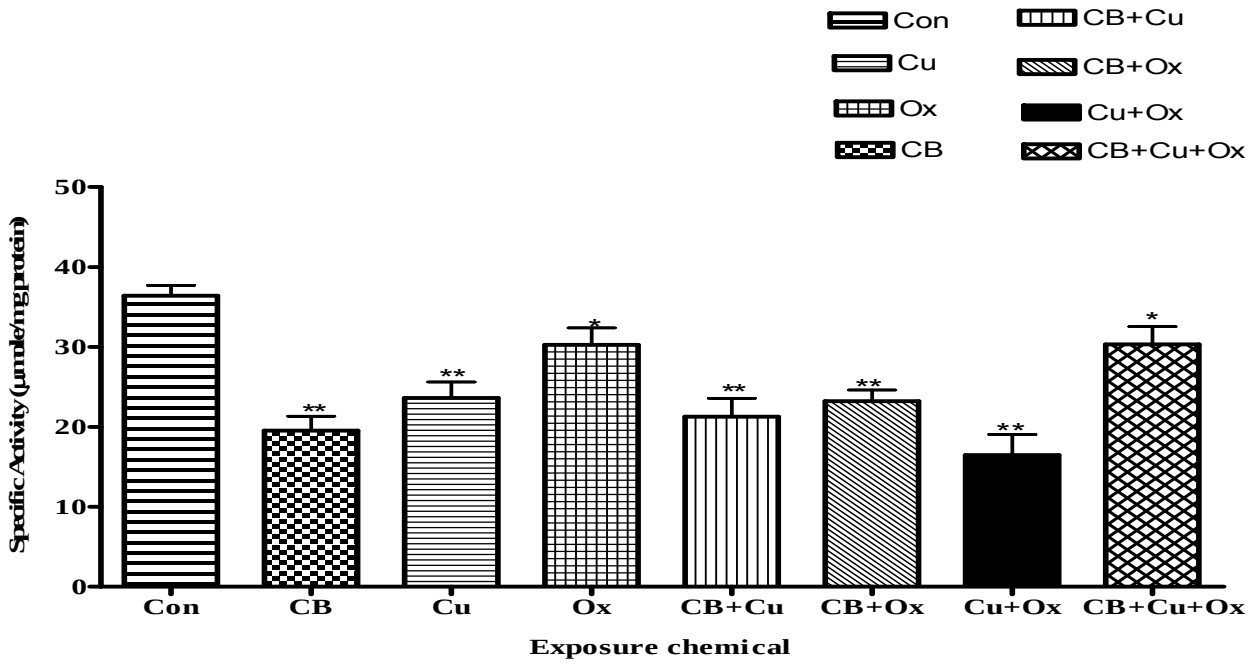

Figure 1. Effect of Con CB Cu Ox , CB+Cu , CB+Ox , Cu+Ox and CB+Cu+Ox, on cholinesterase activity of the freshwater snail *H. duryi*. Esterase activity was measured using acetylthiocholine iodide. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (*$P<0.05$; **$P<0.01$).

RESULTS

Cholinesterase activity

Carbaryl, detergent and copper individually significantly decreased cholinesterase activity in whole tissues homogenates of the freshwater snails *H. duryi* ($p<0.05$ or $p<0.01$). Carbaryl caused the highest inhibition of 46% while the detergent oxyfoam caused the least inhibition 17%. Inhibitions of 42, 36 and 55% were observed when the snails were exposed to the following binary mixtures: carbaryl and copper, carbaryl and detergent and copper and detergent respectively. The mixture of all three pollutants caused 17% inhibition of cholinesterase activity, Figure 1.

Carboxylesterase activity

All three chemicals studied individually caused inhibitions of carboxylesterase activity. Carbaryl and copper caused similar inhibition of carboxylesterase activity (47 and 49%) respectively, the detergent reduced carboxylesterase activity in exposed snails by 15%. The pollutant mixture of carbaryl, detergent and copper caused an inhibition of 22% suggesting antagonistic interactions of the chemical pollutants, Figure 2.

Superoxide dismutase activity

Superoxide dismutase activity was increased in all chemical exposed snails with the highest increase being observed in snails exposed to the metal, copper (133%). Carbaryl and detergent oxyfoam caused inhibitions of 67 and 83% respectively. More than additive effects of the chemicals were observed in snails exposed to the triple mixture of chemicals with an increase of 317%, compared to the added effects of individual pollutants, Figure 3.

Catalase activity

All three chemicals individually enhanced the activity of catalase. The highest activity of individual effects of the chemical pollutants was caused by carbaryl (55%) while copper ions caused the least increase (27%) in catalase activity. Of the binary mixtures, the copper ions and the detergent caused the highest increase in catalase activity 109%. The mixture of all three pollutants increased catalase activity by 123%, Figure 4.

Glutathione peroxidase

Glutathione peroxidase activity was increased in all chemical exposed snails. The chemical mixtures caused

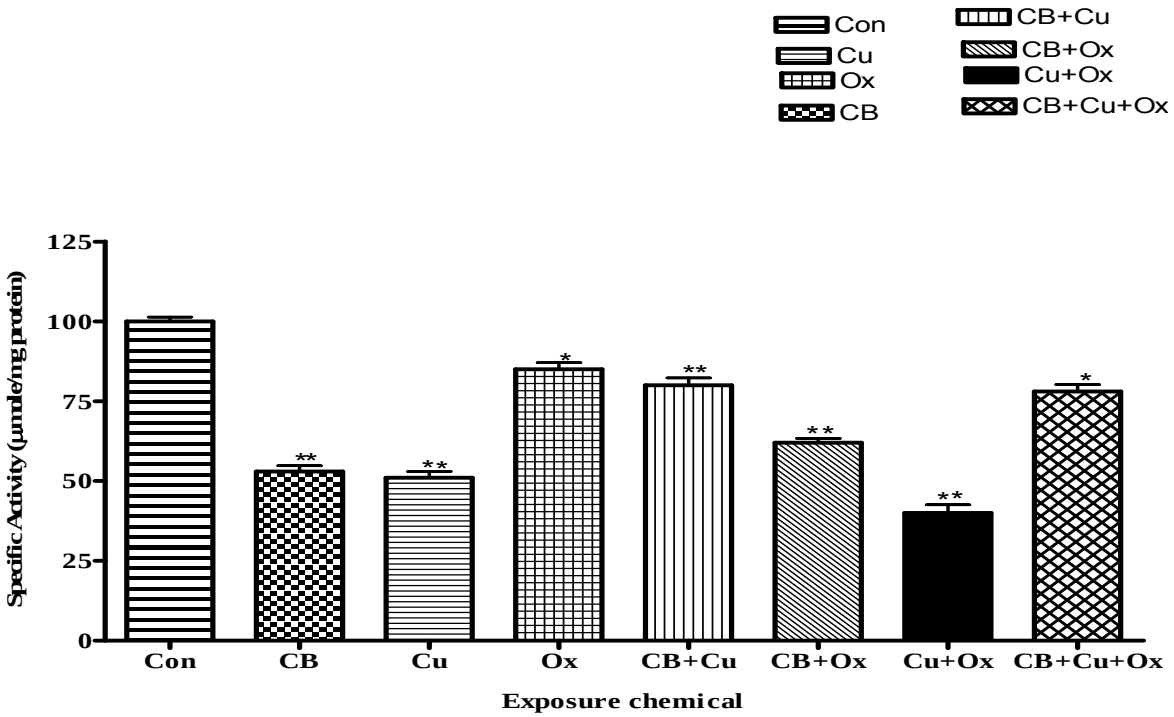

Figure 2. Effect of Con ,CB , Cu , Ox , CB+Cu , CB+Ox , Cu+Ox and CB+Cu+Ox , on carboxylesterase activity of the freshwater snail *H. duryi*. Esterase activity was measured using 4-nitrophenyl acetate. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (**P<0.05*; ***P<0.01*).

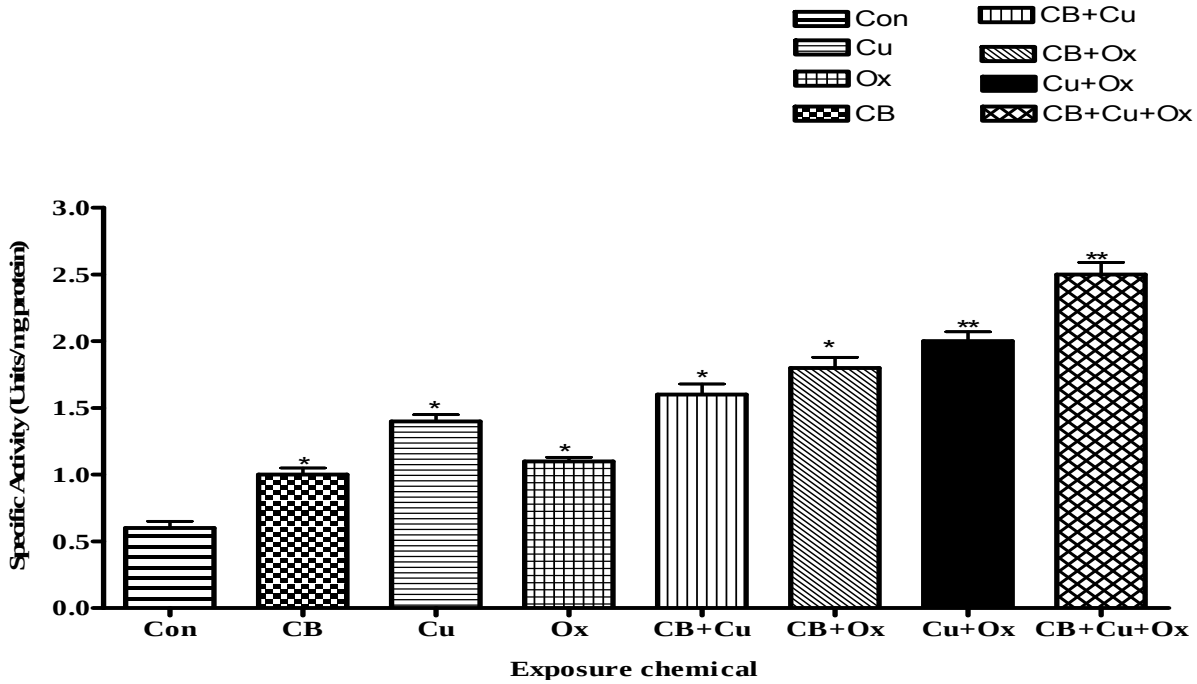

Figure 3. Effect of Con CB u x CB+Cu CB+Ox Cu+Ox and CB+Cu+Ox on superoxide dismutase activity of the freshwater snail *H. duryi*. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (**P<0.05*; ***P<0.01*).

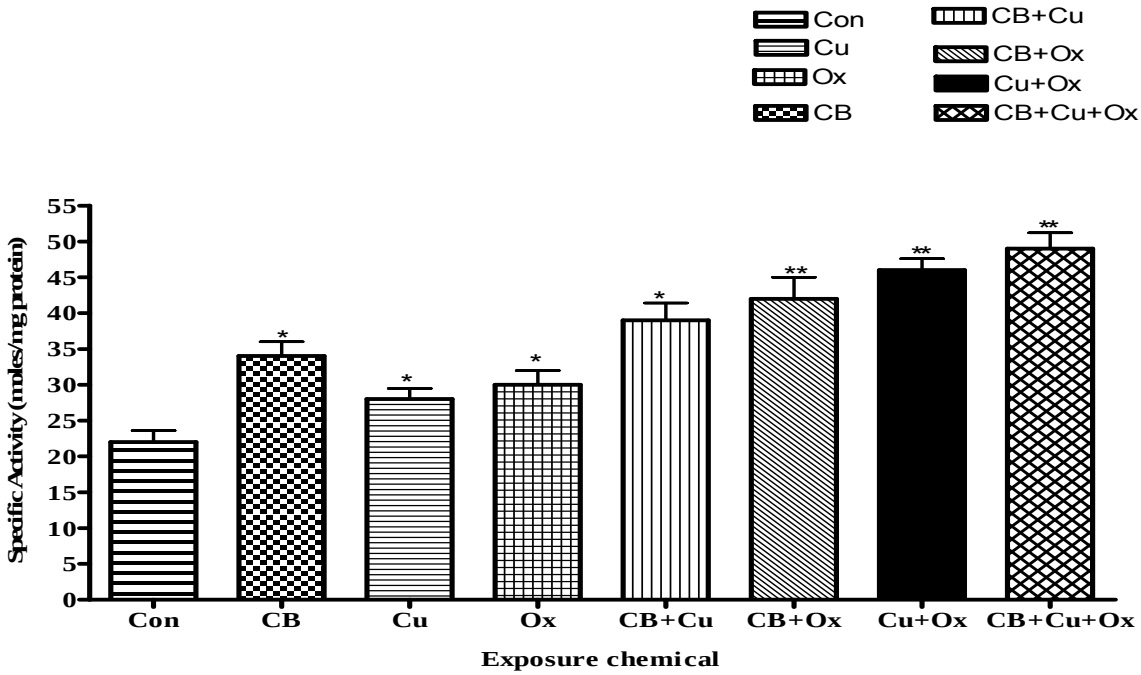

Figure 4. Effect of Con ,CB , Cu , Ox , CB+Cu ,CB+Ox , Cu+Ox and CB+Cu+Ox , on catalase activity of the freshwater snail *H. duryi*. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (*$P<0.05$; **$P<0.01$).

increases in glutathione peroxidase activity which was greater than the added effects of individual chemicals. The triple mixture of pesticide, metal ions and detergent enhanced glutathione peroxidase activity by 260% (Figure 5).

Glutathione S-transferase

All chemicals both individually and as mixtures caused increases in glutathione S-transferase activity. Of the chemical mixtures, the detergent and metal mixture caused the least increase in glutathione S-transferase activity (135%) while the highest increase of 245% was caused by the triple mixture of the chemicals comprising of a carbaryl, copper ions and oxyfoam (Figure 6).

DISCUSSION

Anthropogenic activities release hundreds of chemicals which pollute water bodies such as ponds, lakes and dams. These chemical stressors adversely affect aquatic organisms which reside in these reservoirs. Inhibition of esterase activity, in particular, inhibition of cholinesterase, is used as a biomarker of exposure to carbamate and organophosphate pesticides. Literature shows the importance of alterations of cholinesterase activity as a biomarker of exposure to anticholinesterase agents in particular, organophosphorus and carbamate pesticides.

A significant reduction (*P<0.05) and **P<0.01) of cholinesterase activity in carbaryl exposed snails was observed, Figures 1 and 2. The study showed a 46% inhibition of cholinesterase activity in carbaryl exposed snails. This is supported by the work of Kriestoff et al. (2010) who reported inhibition of cholinesterase activities of two invertebrates, an oligochaete *Lumbricus variegates* and a gastropod *Biomphalaria glabrata* of up to 86 and 65% respectively, after being exposed to the carbamate pesticide carbaryl. Putkome et al. 2008 also observed carbaryl induced inhibitions of up to 90% in molluscs exposed to carbaryl. Carbaryl a wide-spectrum carbamate insecticide, has different formulations which are used for different applications, which include, control of pests that affect citrus fruits, cotton, nuts, poultry, livestock, and other crops.

Carbaryl exhibits its toxicity by disrupting the nervous system; the carbamyl moiety strongly binds with acetylcholinesterase, thereby preventing it from hydrolyzing the neurotransmitter acetylcholine. The build up of acetylcholine results in over excitation of the post synaptic membrane causing paralysis and ultimately death of the insect/pest. Cholinesterase has also been shown to be sensitive to environmental pollutants other than carbamates and organophosphates. Tim-Tim et al. (2009) supports the fact that cholinesterases are

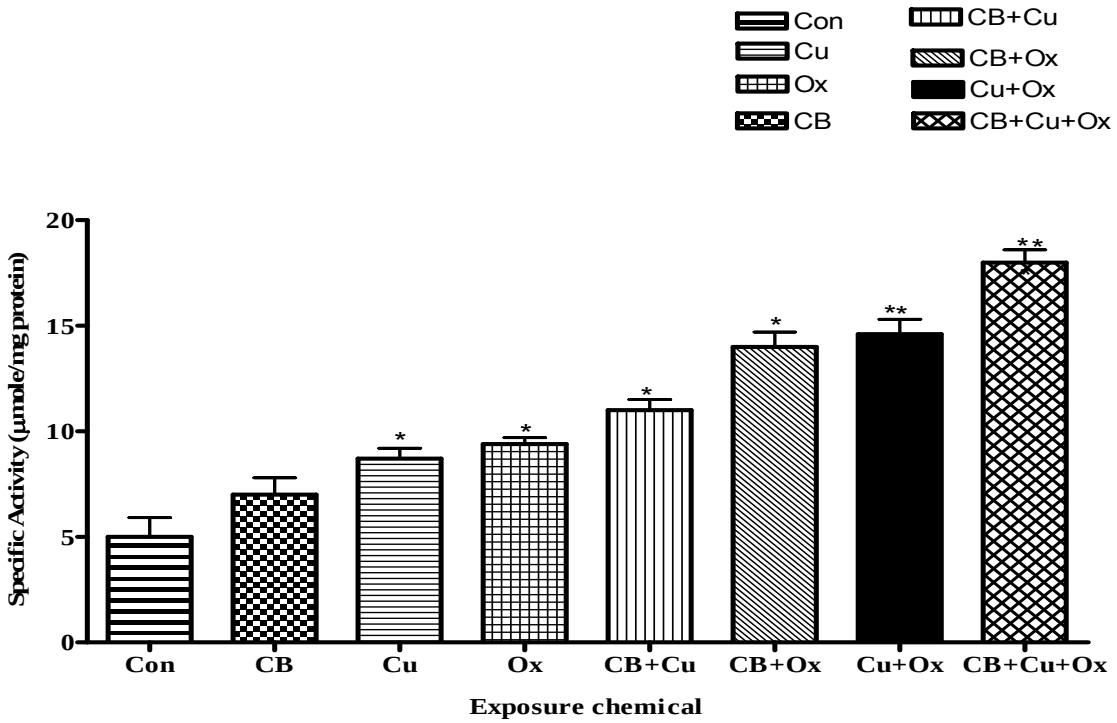

Figure 5. Effect of Con ,CB Cu , Ox CB+Cu CB+Ox Cu+Ox and CB+Cu+Ox on glutathione peroxidise activity of the freshwater snail *H. duryi*. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (**P<0.05*; ***P<0.01*).

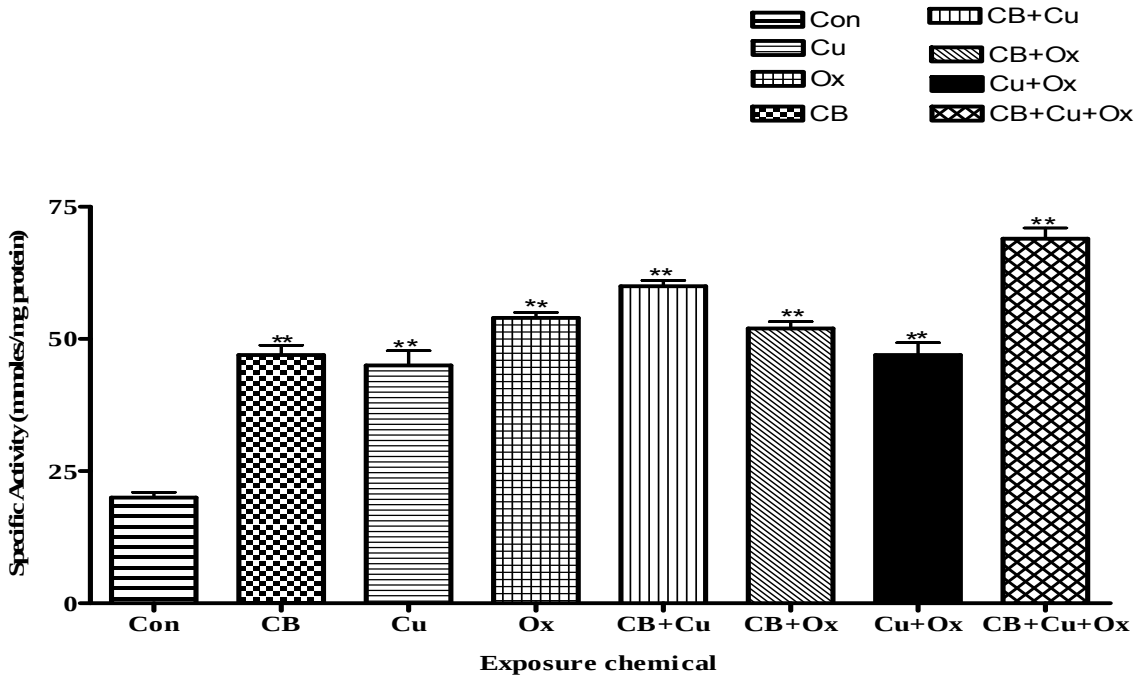

Figure 6. Effect of Con ,CB , Cu , Ox CB+Cu CB+Ox Cu+Ox and CB+Cu+Ox , on glutathione S-transferase activity of the freshwater snail *H. duryi*. CB = carbaryl, Cu = copper, Ox = oxyfoam detergent, CB+Cu = carbaryl and copper mixture, CB+Ox = carbaryl and oxyfoam detergent mixture, Cu+Ox = copper and oxyfoam detergent mixture and CB+Cu+Ox = carbaryl, copper and oxyfoam detergent mixture. Significantly different from control (**P<0.05*; ***P<0.01*).

sensitive to a range of pollutants other than organophosphates and carbamates. The authors reported alteration of cholinesterase activity of three mollusc species exposed to fuel oil.

In the present study, inhibition of cholinesterase activity of the aquatic snails was observed after exposure to the copper ions and a detergent oxyfoam. The results contradict earlier reports in literature (Valbonesi et al., 2003) which specifies cholinesterases as specific biomarkers of exposure to organophosphorus and carbamates pesticides. Reports found in literature differ on how metals affect esterases of different organisms. Romani et al. (2005) observed activation of acetylcholinesterase activity after chronic exposures to sublethal concentrations of copper. Metin et al. (2006) reported enhanced esterase activity in *Geobacillus* sp. after exposure to metals such as nickel and manganese. On the other hand the same authors observed inhibition of esterase activity of *Geobacillus* sp. exposed to copper and mercury.

De-Lima et al. (2013) reported inhibition of acetylcholinesterase activity by *in vitro* exposure to copper, iron, lead and cadmium. In the present study, significant inhibitions of esterase activity of up to 49 and 17% were observed in snails exposed to copper and detergent as individual pollutants respectively, suggesting that the aquatic organisms under study are sensitive to these classes of chemicals even though the chemicals are not designed to target esterases in their mechanism of action. Work on biochemical effects of detergents, in particular, commercial formulations on the well being of aquatic biota is very scarce. Literature reports reveal work done characterizing the properties of detergents however; very little data exists in literature that reports on effects of detergents on esterase activity especially of freshwater snails.

Metin et al. (2006) reported inhibition of esterase activity by ionic detergents while non-ionic detergents cause an opposite effect on the enzyme activity. The composition of the detergent oxyfoam was not determined however our results show that definitely some of the components of the detergent have anti-esterase characteristics indicated by reduction of esterase activity of up to 17% in exposed snails. Inhibition of esterase activity by metals and detergent is probably due to the binding and interaction of both the metal and detergent pollutants with side chains of amino groups that are part of the functional enzymes thereby affecting overall action of the enzymes. Inhibition of esterase activity observed in the current study could also have been due to denaturation of the enzymes caused by interaction of the enzymes with the copper and/or detergent.

Interactions of chemicals in mixtures are complex and difficult to predict, however these interactions affect the overall effects of pollutant to aquatic biota. Generally, the overall effects can be synergistic, additive or antagonistic. Inhibitions of esterase activity in snails exposed to pollutant mixtures were less than the sum effects of individual chemicals implying that there were antagonistic interactions between or amongst the chemical pollutants which affected their overall potency as mixtures.

Pollutants such as heavy metals and detergents have been shown to cause oxidative stress in living organisms. The results of this study showed enhanced activities of SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase) and GST (Glutathione S-transferase) in snails exposed to carbaryl, copper ions and the detergent, individually and as mixtures. The enhanced antioxidant enzyme activity observed was probably an adaptive mechanism by the snails to counteract the oxidative insults in snails caused by exposure to copper, carbaryl and/or detergent. The antioxidant enzymes responded differently to the three different chemical insults. Copper caused the highest increase in the activity of SOD while CAT was affected the most by carbaryl. The activities of the glutathione based enzymes were altered mostly by the detergent oxyfoam.

In the case of mixtures of pollutants, the overall effects observed are determined by the interactions between or amongst the different components of the mixtures. Additive, synergetic and antagonistic effects can be observed depending on the chemical components present. Generally, in this study, enhanced activities were observed implying overall additive effects of the pollutants though the increases in activity ranged from slightly less to slightly higher than additive depending on the antioxidant enzyme measured in both binary and triple mixture exposed snails. Catalase and SOD are considered as the first line of defense against the effects of reactive oxygen species (ROS) in all organisms and as such have evolved in different tissues of both vertebrates and invertebrates (Abdollahi et al., 2004).

All aerobic organisms, aquatic organisms included respond to xenobiotics by biotransforming them to water soluble metabolites which are easy to excrete. However, these metabolic reactions sometimes produce highly reactive oxygen species as byproducts and antioxidant enzymes are induced to deactivate these highly reactive molecules which if not removed may attack the organism's macromolecules leading to tissue damage. Enhanced activities of antioxidant enzymes of aquatic organisms have been associated with efficient defense systems against oxidative stress in organisms. Increases in the activities of antioxidant enzymes CAT (Bianco et al., 2013), CAT and GPx (Guidi et al., 2010), GST (Tim-Tim et al., 2009; Belabed and Soltani, 2013) have been reported in molluscs exposed to different environmental pollutants.

Netpae et al. (2012) support the results observed in the current study, they reported induced activities of SOD, CAT and glutathione reductase in molluscs exposed to copper. Won et al. (2013) also reported induction of SOD and GST genes in polychaetes exposed to copper. Other environmental sources such as domestic and industrial

effluents have also been shown to cause oxidative insults that have resulted in inhibition of antioxidant enzyme defense systems in particular SOD, GPx, CAT and GST. Though literature on toxicological effects of detergents is scant, oxidative stress induced effects of detergents on other aquatic organisms have been reported.

In the current study, enhanced activities of SOD, CAT and glutathione dependent enzymes glutathione peroxidase and glutathione S-transferase were observed in detergent exposed snails. The enhanced enzymatic activities were probably an adaptation defense response to detergent induced oxidative insults. The results are supported by the work of Sobrino-Figueroa (2013) who reported induced antioxidant enzyme systems and high levels of lipid peroxides in detergent exposed fish. Increases in enzyme activity ranges of (55-135%), (27-133%) and (36-170%) after exposure to carbaryl, copper or detergent respectively, depending on the antioxidant enzyme were observed. Mixtures caused higher enzyme activities than those caused by individual pollutants with the triple mixtures showing percentage increases of up to 300% indicating that the antioxidant enzymes of the freshwater snail *H. duryi* are highly sensitive to the studied pollutants.

Bianco et al. (2013) also regards CAT of the freshwater mollusc *Chilina gibbosa* as being sensitive to the organophosphorus azinphos methyl after observing increases of 85% in enzyme activity of exposed snails. Though the pollutant mixtures displayed additive effects on the antioxidant responses of the exposed snails, the observed effects were not mathematical sum totals of the individual pollutants indicating that the interactive effects between and amongst the pollutants definitely have an effect on the overall effects of the pollutants.

There is therefore need to study a wider range of pollutant mixtures to gain a better understanding of chemical reactions that take place amongst pollutant mixture components which affect their overall toxicological effects. Binding of the xenobiotic, metal and/or detergent could have caused the induction of antioxidant defence enzymes by increasing the synthesis and translation of the mRNAs for these enzymes. In the majority of cases, enhancement of activities of antioxidant enzymes system effectively protects the organisms in question from lipid peroxidation and oxidative stress associated with reactive oxygen species such as hydrogen peroxide and free radical molecules. While environmental pollutants normally induce the antioxidant enzyme defense system in exposed organisms as a protective measure against the adverse effects of pro-oxidants, literature has shown that antioxidant defense systems of different aquatic organisms respond differently to environmental pollutants.

Results contradictory to ours which show inhibition rather than induction of the antioxidant enzyme systems are reported in literature. Company et al. (2004) reported Decreases in the activities of SOD, CAT and GPx of molluscs exposed to copper. Nunes et al. (2006) reported reduction in the activities of antioxidant enzymes of crustacean, *Artemia parthenogenetica* exposed to a detergent. The altered activities of the studied enzyme systems of the freshwater snail *H. duryi* observed could be due to the fact that generally, when living organisms encounter foreign bodies they respond metabolically to reduce the adverse effects of the encountered xenobiotic. These metabolic reactions induced to detoxify the xenobiotic may produce reactive oxygen species (ROS) as byproducts which, if not removed can attack the macromolecules causing tissue damage. The antioxidant defense system of the freshwater snail *H. duryi* can potentially be useful tools to detect presence of pollutants such as carbaryl, copper and detergent in aquatic environments.

Conclusion

Pollutant mediated alterations of enzymes systems in aquatic organisms are good indicators of presence of exogenous stressors in aquatic ecosystems and can be used to provide warning signal on the general health status of aquatic biota. Inhibition of esterase and enhancement of antioxidant enzymes activities of *H. duryi* as indicated by our results have potential use as biomarker of exposure to environmental pollutants indicated by the constant response trends observed whether individual or pollutant mixtures were used as environmental stressors.

Conflict of Interests

The authors declare that there was no conflict of interests associated with this study.

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