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Research Article
Carbaryl and Endosulfan Induced Alteration of Biochemical Markers in Soil Organisms, *Eisenia fetida*

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Abstract

Background and Objective: Pesticides are chemicals designed and used to destroy and control pests that affect crops, thereby ensuring food security. Only a small fraction of the sprayed pesticides reach the intended pests, the rest find their way into the environment where they adversely affect non-target organisms. The toxicological effects of pesticide contaminated soil was assessed on selected biomarkers in *Eisenia fetida* to assess whether the pesticides under study have prooxidant properties which negatively impacts non-target soil organisms. **Materials and Methods:** Soil samples were collected from a private farm and a control site. The soils were analyzed for carbaryl and endosulfan. Earthworms were exposed to soils for up to 21 days. The earthworms were then analyzed for antioxidant enzyme activities, reduced glutathione and malondialdehyde levels. Two-way Analysis of Variance (ANOVA) in Tukey's multiple comparison test was used to show statistical differences. **Results:** Carbaryl and endosulfan residues were detected in the soil from the farm. Enhanced activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and DT-diaphorase were observed. Malondialdehyde content was enhanced in earthworms exposed to the soil from the farm compared to malondialdehyde levels in earthworms exposed to the control soil. The activation of antioxidant and xenobiotic enzymes were a result of pollutants in the soil which included carbaryl and endosulfan. Increased malondialdehyde content indicated oxidative stress in the earthworms that were exposed to pollutant-contaminated soils, probably caused by the insecticides carbaryl and endosulfan present in the soil. **Conclusion:** The study highlighted the need of continued monitoring of pollutants in soil to safeguard the health of soil organisms.

Key words: Earthworms, enzymes, pesticides, oxidative-damage, terrestrial-ecosystems, agriculture

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Economies in many developing countries, Zimbabwe included, are built around agriculture. Agriculture depends on pesticides which are chemicals that are used to kill and control insect and plant pests thereby ensuring high quality and quantity yields of crops. Literature studies have revealed that increase in pesticide usage results in increased crop yields per hectare[1]. In Zimbabwe, more than half the population practise farming. Modern farming practices employed by commercial, small-scale and subsistence farmers, exploit agrochemicals that include fertilisers and pesticides. Pesticides in particular insecticides such as organophosphates, carbamates and organochlorines are employed to control pests like aphids, wireworms, armyworms, thrips, mites and cutworms. In Zimbabwe, the broad-spectrum carbamate carbaryl is extensively used to protect several vegetable crops, trees and ornamentals. Mubvereki *et al.*[2] reported in a survey carried out that 10% of the farmers use the acaricide carbaryl in the Checheche, Nemangwe, Sanyati and Tafuna regions in Zimbabwe. Carbaryl also protect apples, sweetcorn, pecans and soybeans[3].

Endosulfan a persistent organochlorine is another pesticide that is used in agriculture in Zimbabwe[2,4]. Endosulfan is a broad-spectrum insecticide that kills a wide range of insects and mites including beetles, aphids, thrips, borers and caterpillars. It has a half-life of 1-8 months in the environment[5]. It is also used for the control of tsetse flies[6]. Its persistence and toxic effects on non-target species in aquatic and terrestrial ecosystems caused its ban as an agrochemical in several countries in the world with a resolution of a complete ban by 2017[7]. However, a number of developing countries, Zimbabwe included still utilize endosulfan for the protection of crops such as cotton[2]. Endosulfan is preferred by local farmers because it is relatively cheap and because of its persistence fewer applications are required compared to non-persistent pesticides like most organophosphates.

While the benefits of pesticide use such as increased food and livestock qualities and yields are significant, the negative effects of pesticides cannot be overlooked. Pesticides contaminate aquatic and terrestrial ecosystems affecting the well-being of aquatic and terrestrial biota[8]. While pesticides reach aquatic systems through leaching, aerial drafts and runoffs, the main receivers of pesticidal pollutants are soils. Undoubtedly soil fauna is chronically subjected to the harmful effects of pesticides. Earthworms are sentinel soil organisms which are ideal organisms to study and gather information about the impact of pesticides in terrestrial environments. Earthworms such as *Eisenia fetida* have important functions in sustaining soil health[9]. They increase soil aeration, nutrient recycling and water movement in the soil[9].

In Zimbabwe even though pesticide use is extensive in all forms of farming, subsistence, small scale and commercial, there is very little information that shows the impact of pesticide use on farmland. This study was designed to investigate the effects of two commonly used pesticides on selected enzymatic markers of the earthworm *Eisenia fetida* to assess the impact of pesticidal use on farms using a private farm in the Matabeleland South region in Zimbabwe as a case study.

MATERIALS AND METHODS

Study area: The study was carried out between 2017 and 2018 at the Ecotoxicology Laboratory, Department of Applied Biology and Biochemistry, National University of Science and Technology, Zimbabwe.

Materials: All chemicals used in the study as well as the pesticide standards, endosulfan (98%) and carbaryl (99.9%) were purchased from Sigma-Aldrich Company. The earthworms were purchased from a local worm breeder.

Sample collection: Samples were collected from Weltevreden, a private farm, in Matabeleland South Province in Zimbabwe. The farming area was divided into five subareas and ten soil samples were collected at random from each subarea following the method described by Namuhani and Cyrus[10]. Soil samples were collected at a depth of approximately 15 and 30 cm wide. The ten samples for each subarea were pooled into a composite sample. The composite samples were analyzed for carbaryl and endosulfan. The soil was also used for exposure studies.

Exposure studies: Earthworms in groups of 18 were exposed to soil from the farm for up to 21 days. Soil in the tanks was kept moist by sprinkling water. The exposures were carried in triplicates, earthworms were subsampled on days 7, 14 and 21. After the exposure periods, the earthworms were sacrificed and analyzed for carbaryl and endosulfan.

Pesticide analysis: Pesticides were extracted from the soil samples using solid-liquid extraction. About 200 mL n-hexane was used to extract the pesticides using a Soxhlet extractor for 3 hrs at 38°C. The samples were then rota-vaporized to 5 mL at a pressure of 2.4 bars at 60°C.

For the earthworms, the pesticides were extracted by homogenization with acetonitrile before being centrifuged at 3000 rpm for 10 min. Clean-up was carried out using a Visiprep vacuum manifold coupled with Oasis HLB 5 cc glass vac cartridge from Waters, Massachusetts, United States of America using 200 mg Sorbent. Carbaryl was eluted using methanol. Endosulfan was eluted using a mixture of hexane/acetone (v/v) in the ration 90:10 using a reverse phase C18 column. The eluted samples were then evaporated to 1 mL using a rotavapor at a pressure of 2.4 bars at 60°C. A method described by Soares *et al.*[11] was followed to determine endosulfan concentration using an Agilent 6820 gas chromatograph equipped with an electron-capture detector (ECD) (Wilmington DE, United States of America). Hexane was used to dissolve endosulfan standards to final concentrations of 20, 50, 100, 200 and 500 ppm[12]. One microlitre of standard/sample was injected in the split-less mode. The oven temperature was set at 80°C, held for 1 min and was ramped at 15°C/min to 180°C. The concentrations of endosulfan in samples were obtained from the standard curve and were expressed as mg/kg.

The concentrations of carbaryl in the samples were measured using an Agilent 6820 gas chromatograph equipped with flame-ionization detector (FID) (Wilmington DE, United States of America) and the carrier gas was nitrogen[11]. Carbaryl standards were dissolved in methanol and the following concentrations were prepared 10, 100, 250, 500 and 1000 ppm[12]. One microliter of standard/sample was injected in the split-less mode. The oven temperature was started at 50°C (held for 1 min) and was ramped at 20°C/min to 100°C, 5°C/min to 150°C hold for 5 min and 10°C/min to 200°C hold for 10 min. The concentrations of carbaryl in samples were obtained from the standard curve and were expressed as mg/kg.

Antioxidant enzymatic markers

Superoxide dismutase activity: The method of Sun *et al.*[13] was used to measure superoxide dismutase activity. In test tubes, 20 µL of post mitochondrial fractions were mixed with 2.45 mL superoxide dismutase reagent and 50 µL of xanthine oxidase and incubated for 20 min. The reactions were stopped by adding 1 mL of 0.8 mM $CuCl_2$. A Shimadzu UV1800 spectrophotometer, (Kyoto, Japan) was used to measure absorbance at a wavelength of 560 nm. The superoxide dismutase reagent contained 0.3 mM xanthine solution, 0.6 mM EDTA solution, 150 µM nitroblue tetrazolium, 400 mM Na_2CO_3 and 1 g/L bovine serum albumin in the ratio 4:2:2:1.2 and 0.6, respectively.

Catalase activity: The method of Claiborne[14] was followed to measure catalase activity. One hundred microliters of PMF was mixed with 1000 µL of 19 mM H_2O_2 in phosphate buffer. Change in absorbance was followed at 240 nm for 30 sec using a Shimadzu UV1800 spectrophotometer, (Kyoto, Japan).

Glutathione peroxidase activity: The method of Flohé and Gŭnzler[15] was followed to analyze glutathione peroxidase activity. The following reagents were allowed to react, 100 µL of 50 mM potassium phosphate buffer, pH 7.0 was added followed by 20 µL of 10 mM reduced glutathione solution, 20 µL of 2.0 mM NADPH in 0.1% $NaHCO_3$ solution, 10 µL of 20 mM sodium azide solution, 20 µL of a 10 U/mL glutathione reductase suspension, 20 µL of the PMF and 10 µL of 2.5 mM hydrogen peroxide. Change in absorbance was followed for 5 min at 340 nm using a SpectraMax 340PC Microplate spectrophotometer, (Sunnyvale, California, United States of America).

Glutathione-S-transferase activity: Habig *et al.*[16] method was used to measure glutathione S-transferase activity. Twenty five microliters of PMF was mixed with 150 µL of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 10 µL of 1 mM sodium azide, 25 µL of glutathione reductase and 25 µL of 10 mM reduced glutathione. Change in absorbance was followed at 340 nm using a SpectraMax 340PC Microplate spectrophotometer, (Sunnyvale, California, United States of America).

DT diaphorase activity: The method described by Lind *et al.*[17] was used to measure diphosphate triphosphate diaphorase activity. One hundred and ten microliters of 50 mM Tris-HCl buffer (pH 7.5) was mixed with 20 µL of 0.8% v/v Triton X-100, 20 µL of 100 µM DCPIP, 30 µL of sample and 20 µL of 5mM NADH. The decrease in absorbance of DCPIP was measured at 600 nm using a SpectraMax 340PC Microplate spectrophotometer, (Sunnyvale, California, United States of America).

Non-enzymatic markers

Malondialdehyde levels: Malondialdehyde levels were measured following to the method of Draper and Hardley[18]. Earthworm tissue samples, 0.2 g each were homogenized in 5 mL of 5% aqueous trichloroacetic acid and 0.5 mL of methanolic butylated hydroxytoluene. The homogenates were then heated for 30 min and cooled to room temperature. The homogenates were then centrifuged at 3000 rpm for 10 min. One milliliter volumes of the supernatants and saturated

thiobarbituric acid were mixed. The mixtures were heated in a boiling water bath for 30 min and then cooled to room temperature. Absorbance was measured at 532 nm using SpectraMax 340PC Microplate spectrophotometer, (Sunnyvale, California, United States of America).

Total Reduced Glutathione (GSH) determination: One hundred milligrams of earthworm tissues were homogenized in potassium phosphate buffer pH 8.0. The homogenates were then centrifuged at 4°C. The PMFs were analyzed for total GSH according to the method of Roušar *et al.*[19]. Ten microliters of 1 mg/mL O-phthalaldehyde solution and 180 µL of the potassium phosphate buffer pH 8.0 were placed in each microtiter plate well. The reaction mixtures were incubated in the dark for 10 min. The fluorescence at an excitation wavelength of 355 nm and emission wavelength of 420 nm was measured using a Shimadzu RF-1501 spectrofluorophotometer (Kyoto, Japan).

Statistical analysis: Data was reported as Mean±SD. Two-way Analysis of Variance (ANOVA) in Tukey's multiple comparison test was used to show statistical differences using the GraphPad prism 7.04 software among the different samples from a commercial farm as well as in comparison with control samples.

RESULTS

Pesticide levels in soil: Residues of endosulfan and carbaryl were observed in soil from Weltevreden farm (Table 1). The detected levels of endosulfan and carbaryl were significantly different ($p<0.05$) to levels of the same pesticides in the control soil.

Pesticide levels in earthworm tissue: Endosulfan and carbaryl residues were observed in earthworms exposed to soil collected from Weltevreden farm were significantly different ($p<0.05$) to levels of the two pesticides in earthworms exposed to control soil (Table 2). The levels of both endosulfan and carbaryl increased with increased duration in exposed snail.

Enzymatic markers

Superoxide dismutase (SOD) activity: The superoxide dismutase activity was enhanced in earthworms exposed to soil collected from Weltevreden Farm compared to the enzyme activity in earthworms exposed to the control soils (Fig. 1). The level of enhancement of SOD increased with increase in exposure duration.

Catalase (CAT) activity: Time dependent increase in catalase activity was observed in earthworms exposed to soil from Weltevreden farm compared to enzyme activity in earthworms exposed to control soils (Fig. 2).

Glutathione peroxidase (GPx) activity: Time dependent increase in glutathione peroxidase activity was observed up to day 14 (Fig. 3). From day 14 to day 28 a decrease in glutathione activity was observed in earthworms exposed to the soil collected from Weltevreden Farm compared to earthworms exposed to control soils.

Glutathione-S-transferase (GST) activity: Time dependent decrease in glutathione S-transferase activity in earthworms exposed to soil Weltervreden farm compared to earthworms exposed to control soil. The lowest GST activity in earthworms exposed to field soil was observed on day 28 (Fig. 4).

DT diaphorase activity (DTD): Diphosphate triphosphate diaphorase (DTD) activity in earthworms exposed to soil from Weltevreden farm was higher than DTD activity in earthworms exposed to the control soil for days 1, 14 and 28 (Fig. 5). Enhancement of DTD activity in earthworms exposed to field soil increased with increase in exposure duration (Fig. 5).

Table 1: Carbaryl and endosulfan residue levels in soil

Pesticide (mg/kg)	Control soil	Field soil
Endosulfan	0.00±0.00	22.24±1.83[a]
Carbaryl	0.00±0.00	9.35±0.67[b]

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

Table 2: Carbaryl and endosulfan residue levels in earthworm tissue

Pesticide (mg/kg)	Control tissue	Tissue day 1	Tissue day 14	Tissue day 28
Endosulfan	0.00±0.00	5.64±0.78[a]	60.73±0.53[b]	70.06±1.28[c]
Carbaryl	0.00±0.00	3.31±0.42[d]	6.49±0.37[e]	9.81±0.54[f]

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

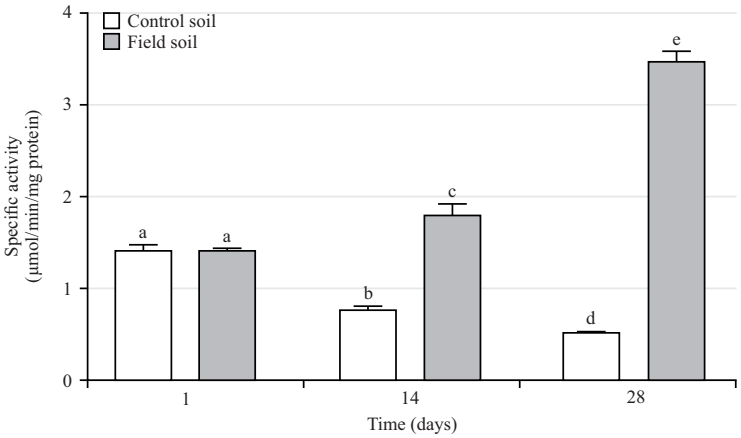

Fig. 1: Superoxide dismutase activity in *E. fetida* exposed to field soil samples from a farm in Matabeleland South Province
Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at (p<0.05)

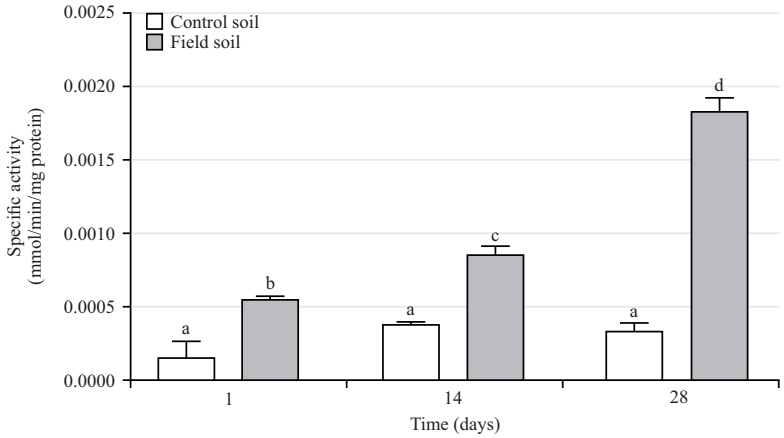

Fig. 2: Catalase activity in *E. fetida* exposed to field soil samples from Weltevreden Farm in Matabeleland South Province
Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at (p<0.05)

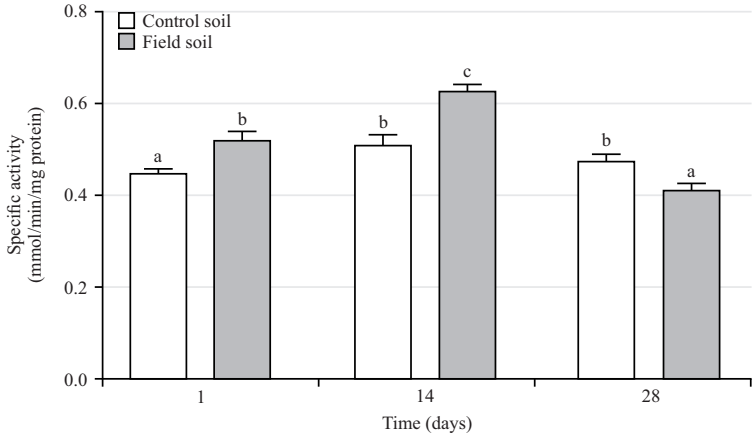

Fig. 3: Glutathione peroxidase activity in *E. fetida* exposed to field soil samples from Weltevreden Farm in Matabeleland South Province
Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at (p<0.05)

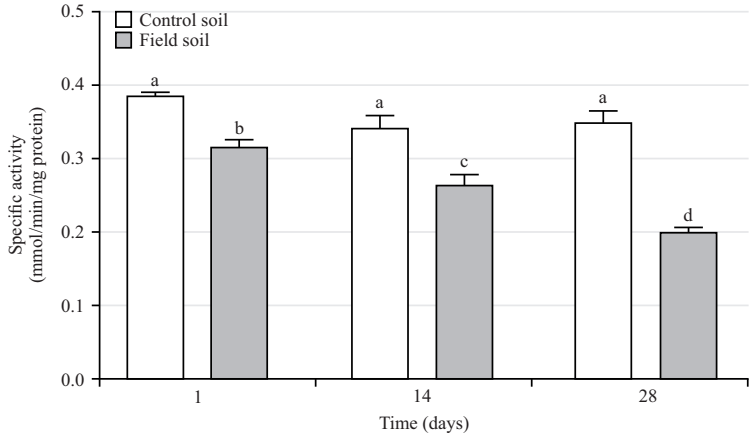

Fig. 4: Glutathione S-transferase activity in *E. fetida* exposed to field soil samples from Weltevreden Farm in Matabeleland South Province

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

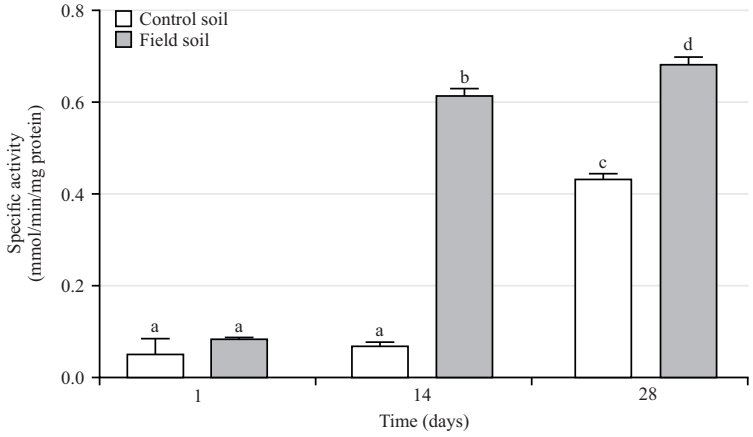

Fig. 5: Diphosphate triphosphate diaphorase activity in *E. fetida* exposed to field soil samples from Weltevreden Farm in Matabeleland South Province

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

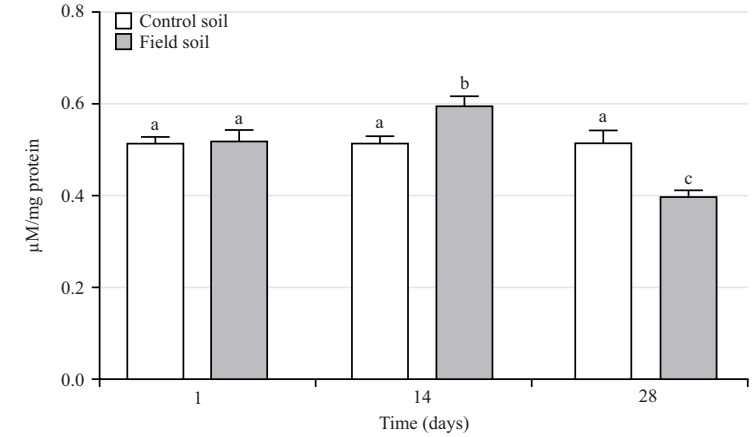

Fig. 6: Glutathione concentration in *E. fetida* exposed to field soil from Weltevreden Farm in Matabeleland South Province

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

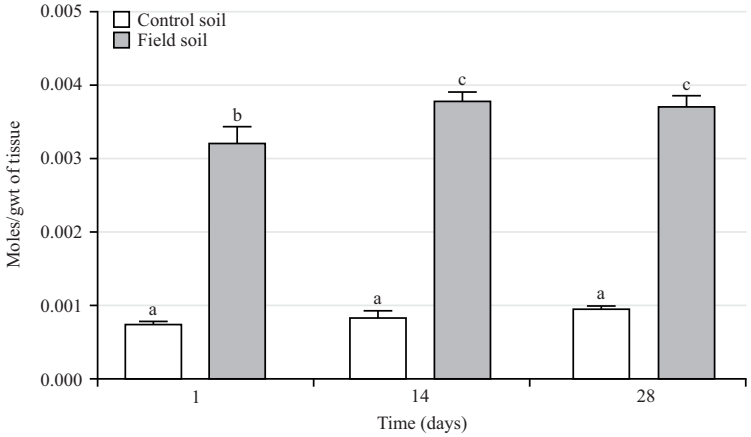

Fig. 7: Malondialdehyde concentration in *E. fetida* exposed to field soil samples from Weltevreden Farm in Matabeleland South Province

Values expressed as mean of triplicate measurements±SD and different letters: Significantly different at ($p<0.05$)

Reduced Glutathione (GSH) concentration: The concentration of Reduced Glutathione (GSH) in earthworms exposed to soil from Weltevreden Farm were significantly higher than in earthworms exposed to control soil in organisms exposed for 14 days ($p<0.05$) (Fig. 6). For earthworms exposed to soil from Weltevreden farm for 28 days GSH levels observed were significantly lower than in organisms exposed to control soil ($p<0.05$) (Fig. 6).

Malondialdehyde (MDA) concentration: Malondialdehyde concentrations were higher in earthworms exposed to soil from Weltevreden farm than in earthworms exposed to control soil (Fig. 7). There was no significant difference in the levels of MDA observed in earthworms exposed to soil from Weltevreden farm for 14 days and those exposed to the same soil for 28 days (Fig. 7).

DISCUSSION

Insecticides and herbicides are used to protect crops from pest thereby significantly reducing losses and improving yields for crops such as maize, wheat and rice[20]. Pesticides are important in human and animal health, protecting animals and humans from diseases[21]. They are used to kill insect vectors like *Anopheles arabiensis* in the control of diseases like malaria[21]. Residues of pesticides particularly persistence agrochemicals such as endosulfan, are found in agricultural soil in relatively high concentrations[22]. In 2005 a global agreement resulted in the banning of persistent organic pollutants which are both toxic and bioaccumulative[23].

Literature reports indicate that only half of the pesticides sprayed by farmers to eliminate pests and improve the quality and quantity of their crops reach the intended pests, the rest end up in aquatic and terrestrial environments causing detrimental effects to habitants of these ecosystems[22].

In this study, no mortalities were observed during the period of investigation in which *Eisenia fetida* earthworms were exposed to field and control soil samples obtained from Weltevreden farm. Booth and O'Halloran[24] observed similar results in that no mortalities were observed in *Aporrectodea caliginosa* exposed to pesticide polluted field samples.

In the current study, soil samples from Weltevreden Farm showed significantly higher levels of endosulfan and carbaryl than levels of the pesticides in the control soils. Qu *et al.*[25] also reported endosulfan and its metabolites in various field soil samples in Southeast China. Organochlorine pesticides such as endosulfan take long to degrade and the process is influenced by factors that include pH, temperature and moisture content[26]. Weber *et al.*[27] reported high levels endosulfan in earthworm tissues and they attributed the bioaccumulation of the pesticide in earthworm tissues to physicochemical factors.

On comparing the levels of endosulfan and carbaryl in Weltevreden farm soils, the current study showed much higher levels ($p<0.05$) endosulfan than carbaryl. The lower levels of carbaryl in the farm soil was attributed to its short half-life of 12 days while endosulfan is cited as a persistent pesticide in the environment[25]. The current study revealed time dependent enhancement of the pesticides under study in particular endosulfan in earthworm tissue (Table 2). Astaykina *et al.*[28] also highlighted bioaccumulation of

endosulfan in exposed *Lumbricus terrestris* earthworms. Endosulfan contaminants in terrestrial ecosystems bioaccumulate and biomagnify through food chains because of their persistent characteristic[7]. The results of the present study indicate that endosulfan and carbaryl caused oxidative stress in exposed earthworms indicated by the significant alterations ($p<0.05$) of antioxidant biochemical markers of the soil organisms (Fig. 1-5). Superoxide dismutase, catalase and glutathione peroxidase were enhanced in a time dependent manner in earthworms exposed to soils contaminated with endosulfan and carbaryl insecticides among other things. This is probably because of the prooxidant nature of the two insecticides. Limon-Pacheco and Gonsebatt[29] were in agreement with our results which affirmed the prooxidant feature of organochlorine and carbamate insecticides on aquatic and terrestrial biota. They identified pesticides as a group of chemical agents that act as pro-oxidants and elicit effects in multiple organs[29]. Insecticides affect the wellbeing of various terrestrial and aquatic organisms by producing free radical species that damage macromolecules including proteins, DNA and lipids[30]. Literature has shown that environmental pollutants like agrochemicals alter the physiological and biochemical state of organisms causing stress and marked activations of antioxidant enzyme systems[31]. The perpetual generation of reactive oxygen species causes oxidative stress in living organisms[32]. In the present study, significant time dependent increases ($p<0.05$) in SOD activity in earthworms exposed to soil from Weltervreden farm were observed compared to enzyme activity in organisms exposed to control soils (Fig. 1). Superoxide dismutases (SODs) are enzymes that disproportionate superoxide anion radicals at some of the fastest enzyme rates known[33]. In this study, the activation of SOD activity was probably a protective mechanism exhibited by the earthworms to eliminate the highly reactive superoxide anion radicals which were converted to H_2O_2 by SOD. Wen *et al.*[34] also reported activation of SOD in earthworm, *Eisenia fetida*, exposed to triflumezopyrim a pesticide used to control rice planthoppers.

Catalase activity like SOD activity was significantly increased in earthworms exposed to soil from Weltevreden farm (Fig. 3). Demircia *et al.*[35] also showed an activation of CAT activity in *Gammarus kischineffensis* exposed to a mixture of the insecticides atrazine and endosulfan. Catalases are enzymes that convert hydrogen peroxide produced by the dismutation activity of superoxide dismutase to water and oxygen. Activation of CAT is a defense response exhibited by the arthropod system to counteract the oxidative effects of hydrogen peroxide[35]. The activation of CAT activity we observed in the current study is supported by studies conducted by Marcano *et al.*[36] who observed enhancement of catalase in earthworms exposed to glyphosate for 7 and 21 days.

Our results showed enhancement of GPx in earthworms exposed to soil from Weltevreden farm for 14 days (Fig. 3). Continued exposure of the earthworms beyond 14 days resulted in a decrease in activity (Fig. 3). Significant reduction of GPx activity was noted in earthworms exposed to soil from the farm from 14 days to 28 days (Fig. 3). The enhancement of GPx was an indication of the activation of protective defense system to prevent the oxidative damage of free radicals the earthworms. Activation of glutathione peroxidase in the digestive gland of the garden snail *Cantareus apertus* exposed to carbaryl was an adaptive strategic process within snail to prevent the pesticide induced oxidative damage to the cellular structure of the exposed snail[37]. In the present study the conjugative glutathione S-transferase activity in earthworms exposed to the field soil from Weltevreden Farm decreased compared to the activity of the same enzyme in snails exposed to control soils (Fig. 4). The reductions in GST activity in the exposed earthworms increased with increase in exposure time (Fig. 4). The decrease in GST activity could have been caused by the stable binding of the pesticides to the active site of the enzyme. Similar trends of reductions in activities of glutathione-S-transferases in earthworms exposed to pesticide contaminated soils compared to enzyme activity in the control has been reported by several studies[35,38,39]. Glutathione-S-transferase enzymes catalyse detoxification reactions by conjugating glutathione to xenobiotics like pesticides them soluble enough for excretion[40]. The activities of glutathione S-transferase a phase II enzyme complements the scavenging reactions of antioxidant enzymes by increasing the solubility of products of phase I metabolites and channeling them for excretion.

The diphosphotriphospho diaphorase (DTD), is an oxidoreductase that catalyzes the reduction of quinones to hydroquinones. The activity of DTD increased with increase in exposure duration in earthworms exposed to soil obtained from the farm (Fig. 5). The activation of DTD was probably a protective mechanism adopted by the earthworm defense system to manage the toxic effects of pesticidal xenobiotics encountered by the organism. The observed results of the present study suggested that the detoxification mechanism of the pollutants in the soil from Weltervreden Farm probably generated quinone-like structures which were then reduced

by DTDs. Harunur Rashid *et al.*[41] mentioned induction of DT diaphorase due to exposure to the organochlorine dichloro-diphenyl-trichloroethane.

In the current study levels of the cellular antioxidant, Reduced Glutathione (GSH), significantly increased in the first 14 days ($p<0.05$) in earthworms exposed to field soil collected from Weltevreden farm (Fig. 6). The levels of GSH then significantly reduced ($p<0.05$) in farm-soil exposed earthworms from day 14 to day 28 (Fig. 6). The decrease in GSH levels in earthworms exposed could have been due to their utilization by the glutathione S-transferases in detoxification of pollutants in the soil. Tiwari *et al.*[30] also highlighted the consumption of glutathione during the detoxification of pesticides by the cytosolic enzyme, glutathione S-transferase in earthworms exposed to contaminated soils.

The extent of oxidative damage caused by exposure to soil pollutants including pesticides was measured by quantifying the amount of malondialdehyde (MDA) generated in earthworms exposed to farm soil. In the current study significantly higher content of malondialdehyde ($p<0.05$) was observed in earthworms exposed to soil from Weltevreden farm for organisms exposed for 1, 14 and 28 days compared to the levels of the same compound in earthworms exposed to control soil (Fig. 6). Malondialdehyde is among the terminal products of the peroxidation reactions of fatty acids and is a proven biomarker of oxidative stress in living organisms[42]. Enhanced MDA content has been reported in earthworms exposed pesticide polluted soils[42-44]. The work of Tiwari *et al.*[30] was in agreement with our results. Elevated MDA levels were reported in earthworms, *Eudrilus eugeniae* exposed to pesticide polluted soils[30]. The increase in MDA levels in earthworms exposed to field soil from Weltevreden farm observed in the current may be indicative of cell damage to lipid membranes. Malondialdehyde is the by-product of the reaction between free radicals and unsaturated fatty acids in cellular membranes[45].

The findings of the present study have demonstrated an alteration in the oxidative status of the earthworms that were exposed to pesticide contaminated soil from Weltevreden farm. The significant elevations of the antioxidant enzymes SOD, CAT and GPX as well as the xenobiotic metabolizing enzymes DTD and GST are reflective of the activation of the adaptive defense mechanisms employed by the earthworms to counteract the oxidative stress induced by the soil pollutants. The earthworms exposed to soil from the farm were under oxidative stress indicated by the high levels of malondialdehyde, a final product of lipid peroxidation. While the activation of the antioxidant first line of defense enzymes is an indication of the earthworms' strategy to prevent the toxic effects of free radicals, the high levels of MDA show that the antioxidant defense system failed to effectively eliminate reactive oxygen species in the earthworms and therefore failed to avoid oxidative damage. Current finding showed that soil pollutants including pesticides adversely affect the wellbeing of soil biota like earthworms which has a consequence of affecting the health and fertility of the soil. Current results suggested that pollutants in soil, pesticides included, affect the wellbeing of soil organisms. It is therefore important to routinely monitor levels of pollutants such as pesticides in farming areas to safeguard the health of soil and its biota.

CONCLUSION

The results of the present study revealed the presence of the endosulfan and carbaryl in the soil sampled from Weltevreden farm. The results also demonstrated the persistence and ability of endosulfan to bioaccumulate in tissues of non-target organisms such as the earthworm *Eisenia fetida*. Carbaryl and endosulfan caused oxidative stress in the exposed earthworms indicated by the observed high levels of malondialdehyde a product of lipid peroxidation. Soil pollutants which include pesticides undoubtedly affect the good health of non-target organisms and there is need for routinely monitoring pollutant levels in soil to safeguard the well-being of non-target soil fauna and flora.

SIGNIFICANCE STATEMENT

Spraying of pesticides remains the most effective method of reducing pest infestations in farming operations thereby ensuring quality and quantity of agricultural output. However, a small portion of the sprayed pesticides reaches target pests with the bulk being-carried by aerial drifts to non-target soil organisms. These soil organisms are beneficial members of the agroecosystems, they maintain and naturally fertilize the soil thereby supporting farming activities and should protected. Consistent monitoring of the health status of soil fauna is of paramount importance as it impacts agricultural outputs, hence this study.

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