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# Determination of Total Protein, Superoxide Dismutase, Catalase Activity and Lipid Peroxidation in Soil Macro-fauna (Earthworm) from Onitsha Municipal Open Waste Dump

**J. C. Ifemeje[1*], S. C. Udedi[2], A. U. Okechukwu[1], A. C. Nwaka[1], C. B. Lukong[1], I. N. Anene[1], C. Egbuna[1] and I. C. Ezeude[1]**

*[1]Department of Biochemistry, Anambra State University, P.M.B.02, Uli, Nigeria.*
*[2]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author JCI designed, wrote the protocol and the first draft of the manuscript. Authors JCI, SCU and CBL performed the superoxide dismutase and catalase activity analysis. Authors AUO, ACN and INA carried out the lipid peroxidation analysis. Authors CE and ICE collected the sample, processed it and managed the literature searches. Authors JCI, CE and ICE performed the statistical analysis, interpreted the results, and formatted the final manuscript. All authors read, corrected, and approved the final manuscript.*

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## ABSTRACT

**Aims:** Oxidants are substances toxic in high concentration, but at low doses can stimulate biological activities of living organisms. The effect of oxidants on cells is modulated by multiple interacting antioxidant defence mechanism. The present study evaluated the individual role of interaction of superoxide dismutase (SOD) and catalase in estimating the level of toxicity of municipal solid waste on soil organisms, and the rate of lipid peroxidation by the action of reacting oxygen species.
**Study Design:** Earthworm samples were collected from four different locations grouped into A, B,

**Corresponding author: Email: jcifemeje@yahoo.co.uk;*

C, and D.
**Place and Duration of the Study:** Analysis was conducted at the Department of Biochemistry, Anambra State University, from March 2014 to May 2014.
**Methodology:** Total protein estimation was determined by Lowry method while superoxide dismutase activity (SOD), catalase activity (CAT) and lipid peroxidation concentration was determined spectrophotometrically using Shimadzu UV-160 spectrophotometer.
**Results:** A high increase in superoxide dismutase and catalase activity as a result of corresponding increase in lipid peroxidation was observed in earthworms of groups C and D (21.22±0.54 and 23.74±0.51 respectively) compared to groups A and B (4.88±0.54 and 7.24±0.31 respectively). Based on the above observation, groups A and B can be classified as samples from the dormant portion while groups C and D can be classified as samples from the active portion of the site.
**Conclusion:** The increased level of superoxide dismutase and catalase activity may be a result of increased concentration of reactive oxygen species (ROS) in waste dumps which can cause damage to soil organisms or even death and hence render the soil from the dump site unfit for agricultural purposes.

*Keywords: Biological activity; Eisenia fetida; free radical; oxidant.*

## 1. INTRODUCTION

Exposure of living organisms to contaminated environments can have significant deleterious consequences [1]. Environmentally induced stress frequently activates the endogenous production of reactive oxygen species (ROS) in organisms; most of which are generated as side products of tissue respiration [2]. ROS includes the superoxide radical ($O^{2-}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^-$), all of which affect biomolecules by interacting rapidly and aggressively with polyunsaturated fatty acids, DNA and proteins due to their unstable nature [3,4]. Free radicals and other reactive oxygen species lead to oxidative stress and have long been known to be mutagenic; these agents have more recently emerged as mediators of other phenotypic and genotypic changes that lead from mutation to neoplasia [5,6].

Oxidative stress however, is the imbalance between the production of free radicals and antioxidant defence systems, either because of a defence deficit or an increase in the production of oxygen reactive species [7]. This stress results in changes in the structure-function relationship of some organs, systems, or specialised cell groups [8,9]. Under normal conditions free radicals and other reactive oxygen species are essential for life, because they are involved in cell signalling and are used by phagocytes for their bactericidal action. Cells are protected against the toxic effects of high concentrations of ROS by a balanced level of endogenous enzymatic and non-enzymatic antioxidants. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and *glutathione* peroxidase (GPx) [10]. Superoxide radicals that are generated are converted to $H_2O_2$ by the action of SOD, and the accumulation of $H_2O_2$ is prevented in the cell by CAT and GPx [10]. David [11] reported that for every 10,000 electrons transferred down the respiratory pathway in *Escherichia coli* cells, about three electrons end up on superoxide instead of the proper place.

SOD works by taking two molecules of superoxide, strips the extra electron off of one, and places it on the other. So, one ends up with an electron less, forming molecular oxygen ($O_2$), and the other ends up with an extra electron. The one with the extra electron then rapidly picks up two hydrogen ions to form hydrogen peroxide which the cell must detoxify through the help of CAT [11]. Notably, the reactive oxygen plays a crucial role in the initiation of lipid peroxidation which can cause oxidative damage to various biological macromolecules [12]. Protective mechanisms that could scavenge the peroxides produced by free radicals have evolved within plants to keep these deleterious compounds to a minimum and these include antioxidant enzymes such as superoxide dismutase and catalase [13].

Lipid peroxides that result from hydroxyl radical attack on the important polyunsaturated fatty acids serves as hydroxyl radical markers caused by oxidative stress. The damage from this oxidative stress in fatty acid rich structures, such as cell membranes, results in loss of rigidity, integrity, and permeability [14]. This process is however referred to as lipid peroxidation and can

be determined from the carbonylic compounds called malondialdehyde (MDA) [14]. The MDA is referred to as an index of lipid peroxidation [15].

The earthworm (*Eisenia fetida*) is considered a representant of soil fauna [16]. Earthworms are used as indicators for assessing the potential impact of chemicals on soil organisms [17]. Biomarkers have been measured in earthworms while assessing their exposure to contaminants in the soil through the measurement of antioxidant enzyme activity [18]. *E. fetida* is characterised by its increased sensitivity to chemical agents compared with other species of earthworms [19]. By definition, biomarkers are biochemical, physiological, and histological reactions in organisms that can be used to determine exposure to and/or toxic effects caused by chemicals [20]. The present study utilized biomakers in *E. fetida* to ascertain the level of toxicity in the soil by examining the interaction of superoxide dismutase (SOD) and catalase activities and the rate of lipid peroxidation of *E. fetida* samples collected in four different sites in the same solid dump site located along Onitsha-Owerri road, Anambra State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

Earthworm samples were collected from four different sites (A, B, C, and D) at the Onitsha municipal solid waste landfill near the National Metallurgical Institute along Onitsha-Owerri road, Onitsha, Anambra State, Nigeria (Figs. 1 and 2) using spade and forceps and analysed separately. The landfill serves as a waste dumpsite for the commercial city of Onitsha where refuse of different kinds ranging from wastes from automobile companies, industries, plastics and glass materials are dumped. Soil from the landfill is often used for agricultural purposes. Earthworm samples were collected from four distinct areas in the dumpsite designated as dormant and active sites. Dormant sites are areas where dumping has been suspended designated as A and B, while the active sites are areas where dumping is still taking place, and were designated as C and D. The control earthworm samples were collected from a waste dump - free farmland in Uli, Anambra State. All the samples were preserved in isolation in ice/cold water to prevent enzymatic or microbial activities before taking them to the laboratory for analysis, following the Standard Operating Procedures described by Scientific Engineering Response and Analytical Service [21]. Each of the earthworm was weighed, macerated and mixed with normal saline in the proportion (1 g of earthworm = 10 ml of normal saline) and poured into a sample tube and refrigerated.

### 2.2 Total Protein Estimation

The protein content was estimated in each earthworm sample using the method of Lowry et al. [22]. One gram of the sample was homogenized with 20 ml of 0.5 M NaOH. The homogenate was poured into a centrifuge tube and centrifuged at 3500 rpm for 10 minutes. The supernatant was collected in a tube. To 1.0 ml of the supernatant, 4 ml of distilled water was added to make up to 5 ml. A standard protein solution of 0.2 mg was prepared (bovine albumin serum) in the same manner. Five ml of alkaline solution was added to each tube and then properly mixed and allowed to stand at room temperature for 10 minutes. This was followed by the addition of 0.05 ml of dilute Folin-ciocalteu reagent to each tube and mixed immediately to give a blue colour. The absorbance was read at 750 nm against a blank reagent using Shimadzu UV-160 spectroscopically. The protein concentration in each sample was extrapolated from the standard BSA curve.

### 2.3 Determination of SOD Activity

The activity of superoxide dismutase was determined by the method described by Magwere [23]. This involves measuring the SOD inhibition of the auto-oxidation activity of epinephrine at pH 10.2 and 30ºC. One unit of superoxide activity is defined as the amount of SOD necessary to cause 50% inhibition of epinephrine auto-oxidation. The analysis was performed in 0.02 ml of the sample and 3.0 ml of 50 M $Na_2CO_3$ buffer. This was followed by the addition of 0.03 ml of epinephrine stock solution before taking the absorbance reading at 480 nm for 3-5 minutes. A blank devoid of the sample (but containing all reagents) was used for background correction.

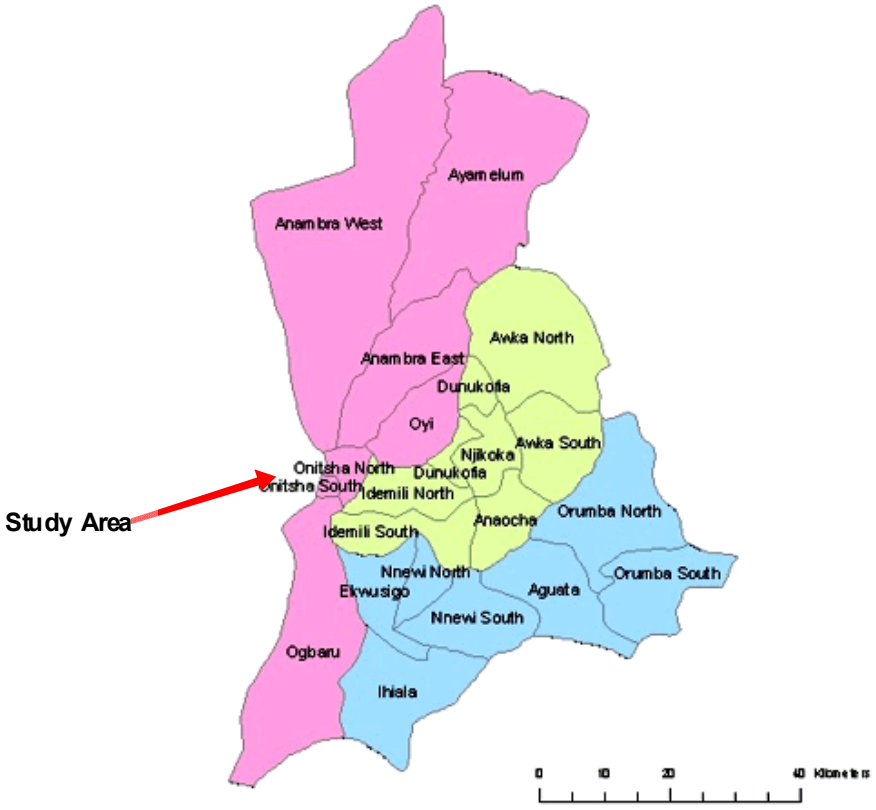


**Fig. 1. Map of Anambra state, Nigeria showing the study area**

**Fig. 2. The municipal solid waste dump site**

### 2.4 Determination of Catalase Activity

Beers and Sizer [24] method was modified forCAT activity assay. A mixture of 2.5 ml of phosphate buffer (pH 7.0), 2 ml of $H_2O_2$ solution and 0.5 ml of sample was added to each tube. The hydrogen peroxide ($H_2O_2$ 30 mM) was used as a substrate and the decrease in $H_2O_2$ concentration at 22°C was measured spectrophotometrically at 240 nm for 1 minute. The activity of the enzyme was expressed in units per mg of protein and 1 unit equals the amount of an enzyme that degrades 1 mM $H_2O_2$ per minute.

### 2.5 Determination of Lipid Peroxidation

The thiobarbituric acid-reactive substances (TBARS) were measured using the modified method of Du and Bramlage [25]. An aliquot of 1.0 ml of incubated sample was added to 0.5 ml of 25% (w/v) TCA and 0.5 ml 1% TBA in 0.3% (w/v) NaOH. The tubes were heated at 90-95ºC in a water bath for 40 minutes and then cooled in ice to reduce turbidity. In this modified procedure, the absorbance was measured at 532 and 600 nm to correct for the interference produced by TBARS sugar complexes. Measurements were carried out in a Shimadzu UV-160 spectrophotometer.

### 2.6 Statistical Analysis

The data were expressed as means±S.D. Statistical significance of differences among treatments were determined by the One-Way Analysis of Variance (ANOVA) and covariance, followed by Tukey's pair-wise comparisons for the significant effects.

## 3. RESULTS

Results revealed that there was a significantly higher concentration of protein in the control and groups A and B compared to the concentration of protein present in groups C and D (Table 1).

SOD activity was significantly higher in groups C and D (21.22±0.54 and 23.74±0.51, respectively) when compared to groups A and B (4.88±0.54 and 7.24±0.31, respectively) (Table 2). This could be a result of low activity going on at the dormant site as compared to the active site which has a high activity.

Catalase activity of groups A to D of earthworms increased with simultaneous decrease of protein concentration, as shown in Tables 1 and 3. Catalase activity was higher in group D followed by group C, while the control and group A were the lowest.

**Table 1. Total protein concentration in earthworms of sites A, B, C, and D in a waste dump**

| Groups | Protein concentration (mg/ml) | Protein concentration Mean±SD* [(1)] (mg/ml) |
|---|---|---|
| Control | 13.52<br>13.51<br>13.61<br>13.42 | 13.52±0.08 |
| A | 14.01<br>10.88<br>12.64<br>14.95 | 13.12±1.77 |
| B | 5.06<br>5.93<br>4.07<br>9.56 | 6.15±2.40 |
| C | 2.20<br>1.98<br>1.65<br>1.87 | 1.92±0.23 |
| D | 1.43<br>1.32<br>1.54<br>1.87 | 1.54±0.24 |

*[(1)] * Protein concentration is presented as mean±SD (mg/ml protein) of four determinations; * Difference in mean values are statistically significant at p<0.05*

**Table 2. SOD activity in earthworms from different waste dump sites**

| Groups | Auto-oxidation rate (units/sec) | Percentage inhibition | SOD activity (U/mg protein) | SOD activity* [(1)] (mean±SD) (U/mg protein) |
|---|---|---|---|---|
| Control | 0.07 | 32.5 | 0.65 | 0.65±0.01 |
| | 0.06 | 32.4 | 0.60 | |
| | 0.07 | 32.5 | 0.65 | |
| | 0.08 | 33.3 | 0.70 | |
| A | 0.58 | 235.8 | 4.72 | |
| | 0.53 | 215.5 | 4.31 | 4.88±0.54 |
| | 0.69 | 280.5 | 5.61 | |
| | 0.60 | 243.9 | 4.88 | |
| B | 0.84 | 341.5 | 6.83 | |
| | 0.92 | 374.0 | 7.48 | 7.24±0.31 |
| | 0.92 | 374.0 | 7.48 | |
| | 0.88 | 357.7 | 7.15 | |
| C | 2.53 | 1028.5 | 20.57 | |
| | 2.66 | 1081.3 | 21.63 | 21.22±0.54 |
| | 2.58 | 1048.8 | 20.98 | |
| | 2.67 | 1085.4 | 21.71 | |
| D | 2.78 | 1130.1 | 22.60 | |
| | 2.90 | 1178.9 | 23.58 | 23.74±0.51 |
| | 2.84 | 1154.5 | 23.09 | |
| | 2.92 | 1187.0 | 23.74 | |

*[(1)]* SOD Activity is presented as mean±SD (U/mg protein) of four determinations; * Difference in mean values of SOD activity are statistically significant at p<0.05*

**Table 3. Catalase activity in earthworm from different sites in a waste dump**

| Groups | Catalase velocity constant (K) | Catalase activity/mg protein | Catalase activity (mean±SD) |
|---|---|---|---|
| Control | 0.01 | 0.001 | 0.001±0.00 |
| | 0.01 | 0.001 | |
| | 0.01 | 0.001 | |
| | 0.01 | 0.001 | |
| A | 0.02 | 0.001 | |
| | 0.02 | 0.001 | 0.001±0.00 |
| | 0.02 | 0.001 | |
| | 0.02 | 0.001 | |
| B | 0.01 | 0.002 | |
| | 0.02 | 0.003 | 0.003±0.00 |
| | 0.01 | 0.003 | |
| | 0.02 | 0.002 | |
| C | 0.05 | 0.022 | |
| | 0.05 | 0.027 | 0.028±0.00 |
| | 0.05 | 0.031 | |
| | 0.06 | 0.030 | |
| D | 0.04 | 0.028 | |
| | 0.06 | 0.042 | 0.036±0.01 |
| | 0.06 | 0.038 | |
| | 0.06 | 0.034 | |

*[(1)] * CAT Activity is presented as mean±SD (U/mg protein) of four determinations; *Difference in mean values of catalase activity are not statistically significant at p< 0.05*

Results also revealed that the lipid peroxidation concentration in groups C and D (6.09±0.16 and 6.73±0.18, respectively) was high compared to groups A and B (3.06±0.06 and 3.38±0.10). The differences were significantly high ($p<0.05$). This result depicts that groups C and D were the active sites while group A and B were the dormant sites.

Results in Fig. 3 showed that SOD and CAT activities and lipid peroxidation concentration were higher in earthworms from active sites (C and D) compared to earthworms from dormant sites (A and B). The values in earthworms from control were the lowest. Differences in these values could be a result of differences in dumping activity at various sites.

## 4. DISCUSSION

Analysis of municipal solid waste dumps based on the activity and composition of earthworms to decompose the waste, dominated by *E. fetida,* revealed that some sites were dormant while some were active. Dormant sites indicated low catalase and superoxide dismutase activities with the corresponding low level of lipid peroxidation reaction in comparison to the active site with high catalase and superoxide dismutase activities and high lipid peroxidation. The result of protein concentration determination (Table 1) revealed that there was low concentra Rtion of protein in groups C and D in comparison with the concentration of protein present in the control, and groups A and B. This suggests that ROS activity could have negatively impacted the protein concentration by increasing the oxidative stress in earthworms from groups C and D thereby leading to depletion of their protein concentration. This result also reflects the low superoxide dismutase activity observed in the control sample (0.65±0.01 U/mg protein), and groups A (4.88±0.54 U/mg protein) and B (7.24±0.31 U/mg protein) as compared to group C (21.22±0.54 U/mg protein) and D (23.74±0.51 U/mg protein) with high SOD activities (Table 2). This suggests a high oxidative stress in active sites C and D when compared to the fact that the damaging effects of ROS were counteracted by the anti-oxidant enzymes which removed the ROS, thereby restoring the balance and protecting the organism from the stress [26]. Similar results in catalase activities (Table 3) were observed in the control (0.001 U/mg protein) and dormant sites A and B (0.001±0.00 U/mg protein and 0.003±0.00 U/mg protein, respectively) compared to that of active site C and D (0.028±0.01 U/mg protein and 0.036±0.01 U/mg protein, respectively).

Lipid peroxidation activity (Table 4) in groups A (3.06±0.06) and B (3.38±0.10) were relatively high when compared to the control (0.12±0.00), but highest concentrations were found in groups C (6.09±0.16) and D (6.73±0.18) at the active site of the solid waste dump. These results are consistent with that reported by Oni and Hassan [26] who observed that there was a higher lipid peroxidation activity in exposed worms versus the control, and that there was elevated malon-dialdehyde (MDA) level in the test soils compared with the control. Thus, elevated level of lipid peroxides strongly suggest hydroxyl radical activity (Fig. 3), and reflect oxidative damage [27]. Saturated fatty acids undergo less peroxidation than their unsaturated counterparts.

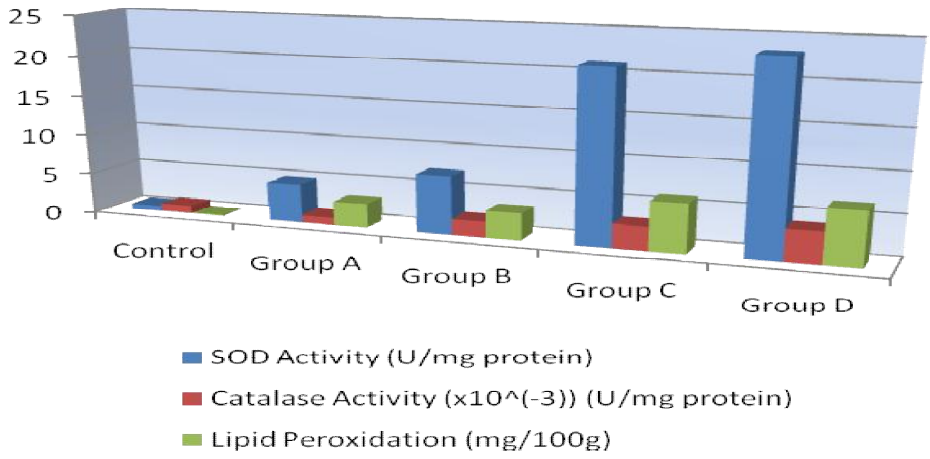


**Fig. 3. Overall comparison of SOD activity, CAT activity and lipid peroxidation concentration in earthworms from each waste dump site**

**Table 4. Lipid peroxidation concentration in earthworms from different waste dump sites**

| Groups | $A_{532\text{-}600nm}$ | Concentration (mg/ml) | Lipid peroxidation (mg/100 g) | Lipid peroxidation,* [(1)] (mean±SD) (mg/100 g) |
|---|---|---|---|---|
| Control | 0.051 | 0.012 | 0.12 | 0.12±0.00 |
| | 0.054 | 0.014 | 0.14 | |
| | 0.050 | 0.010 | 0.10 | |
| | 0.051 | 0.012 | 0.12 | |
| A | 1.225 | 0.299 | 2.99 | |
| | 1.282 | 0.313 | 3.13 | 3.06±0.06 |
| | 1.243 | 0.304 | 3.04 | |
| | 1.252 | 0.306 | 3.06 | |
| B | 1.404 | 0.343 | 3.43 | |
| | 1.348 | 0.330 | 3.30 | 3.38±0.10 |
| | 1.432 | 0.350 | 3.50 | |
| | 1.347 | 0.329 | 3.29 | |
| C | 2.418 | 0.591 | 5.91 | |
| | 2.577 | 0.630 | 6.30 | 6.09±0.16 |
| | 2.470 | 0.604 | 6.04 | |
| | 2.498 | 0.611 | 6.11 | |
| D | 2.838 | 0.694 | 6.94 | |
| | 2.779 | 0.679 | 6.79 | 6.73±0.18 |
| | 2.680 | 0.655 | 6.55 | |
| | 2.708 | 0.662 | 6.62 | |

*[(1)] * Lipid peroxidation is presented as mean±SD (mg/100 g) of four determinations; *Difference in mean values of Lipid peroxidation are statistically significant at p<0.05*

This could be dangerous because unsaturated fatty acids are mainly essential fatty acid needed for living systems to function maximally. Indeed, supplementation with polyunsaturated as opposed to saturated fatty acids results in a statistically significant increase in lipid peroxidation in the plasma and liver [28-30]. However, the study clearly shows the effects of contaminated dumpsite soils on *E. fetida.*

From above it can be deduced that refuse dumping could lead to the generation of reactive oxygen species, i.e, free radicals and this may render soil from the dump site which may be rich in nutrients unfit for agricultural purposes [31]. The prevention of oxidation is an essential process in all aerobic organisms, as decreased antioxidant protection may lead to cytotoxicity, mutagenicity and / or carcinogenicity [32]. To adapt to this effect, earthworms migrate to safer places which subsequently leads to loss of their beneficial functions in soil aeration, drainage, enrichment of organic material leading to loss in soil fertility. The food chain in the dumpsite is also affected because there could be decrease in the numbers and distribution of their vertebrate predators [26]. Thus the loss of earthworms due to toxicity from an area can impact on ecosystem [33].

## 5. CONCLUSION

The exposures of living organisms to municipal solid waste dump sites could be deleterious to health due to the damaging effects of ROS. Living systems such as in the earthworms tends to use their defence mechanisms to annul the changes. Therefore, it is conceivable that soil organisms could suffer a significant mortality when exposed to high content of municipal solid waste. Further study on different dump sites should be carried out to ascertain the level of toxicity. Measures to check this effect will also be investigated.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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