EVALUATION OF THE EFFECT OF BIOCHEMICAL MARKERS AND CATALASE ACTIVITY GENERATED BY AN HERBICIDE (LINURON) IN EARTHWORMS *LUMBRICUS TERRESTRIS*, SOUK-AHRAS AREA, NORTH-EAST OF ALGERIA

BERROUK, H.[1*] – BOUKEHILI, K.[1] – BAKKAR, I.[1] – BOUZNED, H.[1] – KHALDI, F.[2]

[1]*Laboratory of Aquatic and Terrestrial Ecosystem, Department of Fundamental Studies in Biology, Faculty of Life and Natural Sciences, Mohamed El-Cherif Messaadia University, Souk-Ahras 41000, Algeria*

[2]*Laboratory of Sciences and Technology of Water and Environment, Mohamed El-Cherif Messaadia University, Souk-Ahras 41000, Algeria*

**Corresponding author*
e-mail: houda.berrouk@yahoo.fr, h.berrouk@univ-soukahras.dz

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Abstract. Earthworms are ecosystem engineers, owed to their ability to modify the soil resources for other organisms through induction of important changes in the state of the biotic and abiotic soil components. They are very sensitive small invertebrate animals to agricultural pesticides, as well as the best soil pollution bio-indicators. Thus, the present study aimed to assess the effects of an herbicide (Linuron) induced changes in major biochemical markers (the content of total protein, total carbohydrate, total lipid, and catalase activity) in *Lumbricus terrestris*. Statistical analysis of data revealed significant differences in the content of total proteins, lipids, and total carbohydrates, and no significant difference in catalase activity between control and Linuron-treated *L. terrestris* over the three exposure periods (24 h, 48 h, and 72 h) and the three doses used (250 µg, 500 µg, and 1000 µg). Moreover, correlation coefficient analysis showed a positive correlation between protein and lipid contents (r = 0.50, r2 = 50%) during all three exposure periods. Conclusively, this study suggested the determined metabolites as a valuable tools in the environmental risk assessment, and the understanding of the process underlying Linuron induced oxidative stress in *L. terrestris.*
Keywords: *Lumbricus terrestris, bio -indicator, herbicide, linuron, metabolites, catalase*

Introduction

The increased industrial and agricultural human activities have introduced into the environment a considerable amount of hazardous contaminants (Pretty et al., 2015). Among these, pesticides that initially appeared to be beneficial, but their uncontrolled uses can induce harmful effects on living organisms. In addition, long-term exposure to pesticides can directly infiltrate into the depths of the soil and groundwater, and consequently cause serious threats to plants, animals, and human health (Bahandri et al., 2020). Due to pesticides, in particular, herbicides, in recent years have a worldwide use in modern agriculture to increase the yield of many necessary crops, since the residue analysis of these herbicides was not systematically carried out (Redriguez et al., 2018; Pretty et al., 2015). Maksyniv (2015) reported that 95% of herbicides have been identified as other than pathological weeds, and thus they may affect non-target species like earthworms, or may be integrated into food webs and, eventually undergo bio magnification in food chains (Martin et al., 2018). A large amount of available toxicological data reporting the impact of pesticides on human and environmental health require great caution in their use (Multigner, 2005). Moreover, Linuron named as Herbasate or Etalon, is a foliar and systemic herbicide, highly effective on all weeds,

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and can be absorbed by the leaves and carried by the sap to the shoot and root tips. It inhibits weed photosynthesis by good selectivity and an efficient duration of 3 to 8 weeks supported by sufficient humidity. Also, Linuron, a plant protection product, has been reported to have a serious irreversible oral exposure effect on humans, including likely fertility, pregnancy toxicity, and carcinogenic effects, in addition to long-term harmful effects on aquatic environmental organisms. As a result, a large scientific community, in recent years, has made a great effort in developing the monitoring tools of environmental quality risk assessments (Reinecke et al., 2007). Although the physicochemical analysis methods are still the most effective tools for assessing the environmental risks of pesticides on living organisms, they need to be supplemented with biological and biochemical data to assess the potential and overall effect of these products present in the various organs of living beings and the different ecosystem compartments (Denoyelle et al., 2007; Redriguez et al., 2018). Thus, it seems that the use of animal models as environmental disturbance indicators based on their biochemical, physiological, and ecological profile changes is highly important to study the level of pesticides that contaminate the environment (Pretty et al., 2015). The metabolic energy reserves such as proteins, carbohydrates, and lipids provide effective knowledge of the organism's ability against natural or anthropogenic stress. These reserves are, therefore, good biomarkers of the organism and environment health, and the first organic nutrient reserves that can be broken down in response to any environmental stress (Connell et al., 1999; Smolders et al., 2005). Further, catalases are peroxisomal enzymes inhibiting hydrogen peroxide-induced peroxidation of biological molecules, and are highly sensitive to pollutants such as pesticides and heavy metals, whereas their increased level explains the destruction of animal cells following pollutants exposure. Earthworms are soft-bodied invertebrates playing an important role in the development and maintenance of soil fertility, as they dominate the biomass of most terrestrial ecosystems. Also, they represent a major component of the soil macro fauna and are essential to the survival of all soil inhabitants by decomposing and transforming organic wastes into nutrient-rich vermicompost and ingesting huge quantities of soil, and they are highly exposed to various harmful substances (Lavelle and Spain, 2001; Jansirani et al., 2012; Berrouk et al., 2023). Consequently, earthworms are considered a key taxon for assessing the risks associated with the use of plant protection products, and the best monitors provide important knowledge on the impact of pesticides and various pollutants contaminating the terrestrial environment (Abdallah, 2012; Le Bayon et al., 2016; Berrouk et al., 2022). As the interactions between earthworms and different types of pesticides have been previously well studied in many soils around the world, the present study was undertaken to investigate the effect of an herbicide named Etalon, widely used by farmers in the Souk-Ahras city, north-east Algeria, on a biological invertebrate model of the earthworm family *L. terrestris*, omnipresent in the north-east Algeria. The research is based on studying the variations in the essential metabolites and catalase enzyme activity, as well as to demonstrate the toxicity of Linuron as a preferred herbicide acting against weeds.

Materials and methods

The study site

The study site, where the earthworms were obtained, was a house garden where the soil is normally healthy, because we do not use pesticides, located in the region of Sidi-

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Fredj, municipal of Lemrahna of 40 km from Souk-Ahras city (North-East Algeria) with a National Statistics Office: NSO code of 4120, a geographical coordinates of 36’ 9’ 13’ North and 8’ 11’ 43’ East, and a Mediterranean climate with a hot summer and a markedly cold winter (Journal official, 1984) (*Fig. 1*).

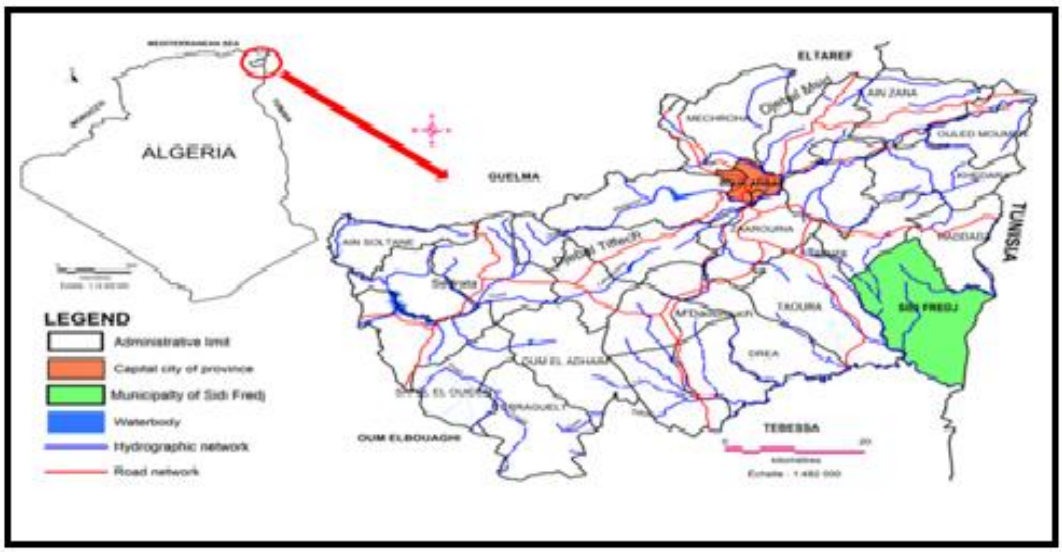

Figure 1. *Sampling site (Sidi Fredj, Souk-Ahras city, Algeria) (Berrouk et al., 2022)*

Soil physicochemical analysis

The present study was performed at the same time, in the same region and with the same species as a previously reported by Berrouk et al. (2022) study using the fungicide Mikal-Flash, where the physico-chemical analysis of the original soil proved its richness in organic matter (16. 12%), a clay-loam texture and moisture content (50.90%) ranging from 45 to 60%, moderately richness in CoCO3 with levels ranging from (10-25%), total carbon concentration of 9.37%, slightly alkaline values of pH water and pH KCl between 7.1 and 8.0, and very richness in phosphorus content with a level above 20 ppm.

Animals

The study was conducted on earthworms *Lumbricus terrestris* (Linnaeus1758) due to their low costs, easy handling, and rapid adaptation to laboratory conditions, and thus is a good biological model for toxicological research. Earthworms were collected from Sidi-Fredj in Souk Ahras city (Northeast of Algeria) by the physical hand method as previously described by Bouché (1972). This method is based on digging the soil to a depth of 20 cm, where the earthworms are inhabited; the samples were afterward transported to the laboratory in air-tight boxes and acclimatized to laboratory conditions for 14-21 days in the soil of their natural habitat.

Chemical

Linuron ($C_9H_{10}C_{l2}N_2O_2$) is a selective systemic herbicide of the substituted urea family, commonly used in Algeria for pre- and post-emergence weed control by inhibiting photosynthesis. It contains a 50% active ingredient, ‘linuron’ (N-3,4-

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Dichlorophenyl)-N'-methoxy-N'-methylurea), and preferably can be used alone without mixing with other herbicides. It is commercialized with a standard dose of 2.5-3 Kg/ha (Technical sheet C.H.P.P.U.A.N 064413).

Methods

In our experimental conditions, Linuron was applied by direct spraying of increasing doses (250 µg, 500 µg, 1000 µg/500 g soil) on the original soil, the choice of doses is according to the doses of Linuron applied to the field, and 120 adult earthworm individuals characterized by a well-identified clitellum were equally divided (30 individuals per tray) into three treated trays and one control tray watered with water. All trays were covered with muslin cloth to facilitate breathing and to prevent the earthworm from escaping. After the exposure periods (Acute toxicity) (24 h, 48 h, 72 h), 9 individuals recovered from each spout (3 repetitions to test the (period-, Linuron effect, and the dose - Linuron effect) (Berrouk et al., 2022; Lordache and Borza, 2011). Our samples were washed with distilled water, weighed, and placed in small plastic tubes containing each 1 ml (50 mM, 7.5 pH) phosphate buffer, and stored in the freezer at -32°C until the biochemical analysis of the metabolites (total proteins, total carbohydrates, total lipids) and the CAT activity, where earthworms must be manually ground using a mortar beforehand (*Fig. 2*).

Figure 2. *Experimental culture*

Metabolite extraction

The earthworm metabolites were extracted according to the experimental protocol previously described (Shibko et al., 1966). In brief, the selected adult earthworms were ground in 1 ml of 20% trichloroacetic acid (TCA), mechanically ground, homogenized with vortex, and then centrifuged at 5000 rpm for 10 min. After that, the supernatant was recovered for total carbohydrate determination, and the pellet was mixed with 1 ml of the ether/chloroform, centrifuged (500 rpm, 10 min) after a second, and the resulting supernatant was used for the determination of total lipids, while the pellet was mixed with 1 ml of distilled water and used for the quantification of total proteins.

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Determination of total protein content

Protein content was quantified using the method of Bratford (1976) in which an aliquot fraction of 100 µl of the supernatant was mixed with 4 ml of coomassie blue dye reagent (BBC) G250, shacked for 2 h, and then mixed with 100 ml orthophosphoric acid 87% topped up to 1000 ml with distilled water). Absorbance readings were taken at a wavelength of 595 nm, in spectrophotometer (Secomam U Viline 9400) and the calibration range was based on using a standard protein (bovine serum albumin BSA) (*Eq. 1*).

$$Y = 0.009x + 0.011 \; R^2 = 0.997 \qquad \text{(Eq.1)}$$

Determination of total carbohydrate content

Carbohydrates content was determined as previously described Duchateau and Florkin (1959). This method is based on the addition of 4 ml of anthrone reagent to 100 µl of the supernatant. The calibration range was obtained using a 1 g/l glucose stock solution as standard. After heating in a water bath (80°C, 10 mm), a green coloration appeared whose intensity was proportional to the concentration of total carbohydrates at a wavelength of 620 nm. The values obtained were plotted against the following sampling *Equation 2:*

$$Y = 0.010x - 0.096 \; R^2 = 0.994 \qquad \text{(Eq.2)}$$

Determination of total lipids content

The total lipids content was determined according to a method previously reported (Goldsworthy et al., 1972), using vanillin as a reagent and a lipid stock solution at 25 mg/ml. After centrifugation (5000 rpm) in centrifuges (Sigma 2-16KL), the supernatants were collected and evaporated to dryness in open tubes for 20 mm, allowed to cool, and then 1 ml of 96% sulfuric acid was added, shaken, and heated to 100°C in a water bath. After the cooling step, a volume of 200 µl was removed from each tube, 2.5 ml of vanillin was added, and the mixture was shaken 30 mm in the dark. The complex turned pink, and its optic intensity was read at a wavelength of 595 nm, in spectrophotometric. The calibration range was carried out using a lipid stock solution, and the obtained values were plotted against the following sampling *Equation 3:*

$$Y = 0.003x - 0.041 \; R^2 = 0.972 \qquad \text{(Eq.3)}$$

Determination of catalase activity

The determination of catalase (CAT) activity was determined as described elsewhere (Claiborne, 1985), based on the variation of the optical density following the disappearance of hydrogen peroxide H_2O_2 at a wavelength of 240 nm. Briefly, earthworms were homogenized in 1 ml phosphate buffer (100 Mm, pH7.4), the homogenate was centrifuged at 15,000 rpm for 10 mm, and the supernatant as a source of the enzyme was kept. For a final volume of 1 ml, the mixture contains 200 µl of supernatant (hydrogen peroxide and 780 µl of phosphate buffer). The reaction was triggered by the addition of hydrogen peroxide. The enzymatic activity was spectrophotometrically determined by following the kinetics reaction for 1 min at 240 nm in quartz UV cuvettes on a spectrophotometer.

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Statistical analysis

The conditions of applying the statistical tests were checked in compliance with the recommendations previously reported (Dagnilie, 2006, 2007; Scherrer, 2007). Data processing and analysis were carried out using a specialized statistical software package, Statistica 8.0, where statistical analysis of the obtained data was performed as follows:

- The statistical description was tested for each studied variable (biochemical and enzymatic), based on the calculation of the mean, the median, and the two minimum values Xmin and maximum Xmax.
- The comparison between multiple groups was tested by the non-parametric Kruskal Wallis test (once to test the "period" effect relative to "Linuron" and once to test the "dose" effect relative to " Linuron ").
- The Spearman correlation coefficient analysis of linear relationships was applied to the various parameters during all the treatment periods used.

Results

Effect of Linuron on the biochemical parameters of L. terrestris as a function of exposure time

As indicated in *Table 1*, Kruskal Wallis test proved marked changes in the most determined biochemical parameters in Linuron -exposed *L. terrestris* as a function of exposure time (24 h, 48 h, 72 h).

Table 1. *Effect of Linuron exposure period on the biochemical parameters in L. terrestris*

	H	***P***
Proteins	12.33	**0.0021***
Carbohydrates	18.28	**0.0001***
Lipids	23.62	**0.0000***
CAT	3.77	0.1518

Data are analyzed by Kruskal Wallis test

Effect of Linuron on total proteins content

As shown in *Table 2* and *Figure 3*, the total protein content (µg/individual) is significantly difference between control and treated *L. terrestris* with the doses (250, 500, 1000 µg/500 mg soil) ($p = 0.0021$) as a function of exposure periods (24, 48, 72 h). Also, the total protein content is higher in treated *L. terrestris* with the three doses after 72 h than that in the control.

Table 2. *Statistical description of protein content during the different treatment periods*

Dose	**Mean**	**Median**	**Min**	**Max**
		24 h		
Control	66.44	76.00	40.33	83.00
D1	48.44	27.55	12.33	105.44
D2	29.33	31.44	21.55	35.00
D3	85.55	66.33	43.55	146.78

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48 h				
Control	16.67	19.89	4.89	25.22
D1	31.52	8.33	2.67	83.55
D2	46.19	36.22	30.22	72.11
D 3	34.70	47.11	2.22	54.78
72 h				
Control	94.62	140.11	2.00	141.78
D1	102.74	139.78	135.78	141.00
D2	136.44	138.11	30.55	139.55
D3	138.85	136.89	132.89	139.55

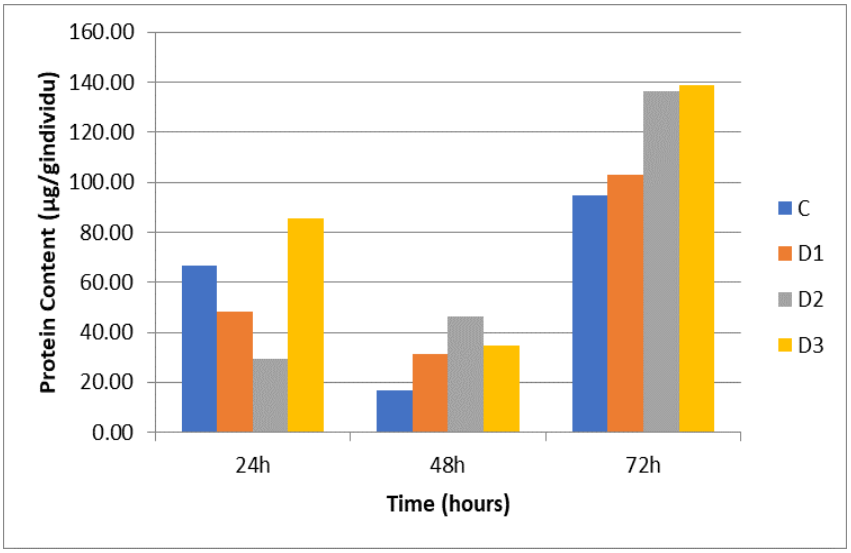

Figure 3. *Effects of Linuron contaminated soil on total protein content (µg/g individual) during different treatment periods. Significant differences (p = 0.0021)*

Effect of etalon on total carbohydrates content

Table 3 and *Figure 4* showed highly significant differences (p = 0.0001) in the carbohydrate concentrations (µg/g individual) between control and treated earthworm at doses (250, 500, 1000 µg/500 mg/soil) as a function of treatment periods (24, 48, 72 h). The carbohydrate content increased in a dose-dependent manner for the three doses compared to the control after 24 h, and for the doses 250 and 500 µg during 48 h, meanwhile its content was markedly decreased in the dose of 1000 µg even after 72 h.

Table 3. *Statistical description of carbohydrate content during the different treatment periods*

Dose	Mean	Median	Min	Max
24 h				
Control	110.72	124.40	107.25	140.55
D1	121.57	109.50	96.25	126.40
D2	124.07	119.00	116.30	129.40
D3	126.38	128.25	112.10	138.80

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48 h				
Control	191.60	226.40	99.40	249.00
D1	213.50	249.90	120.70	269.90
D2	257.27	283.00	190.80	298.00
D 3	182.90	187.60	166.40	194.70
72 h				
Control	152.53	149.90	148.75	158.95
D1	152.22	151.90	146.05	158.85
D2	149.78	150.75	144.25	154.35
D3	127.33	147.65	86.20	148.15

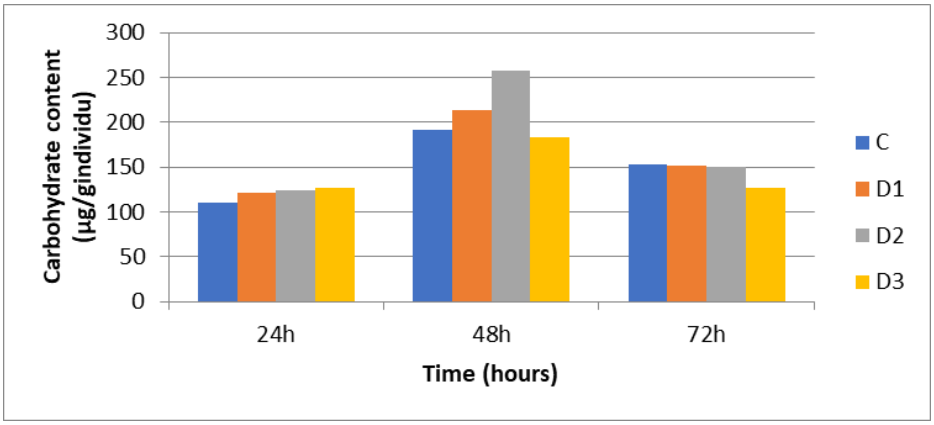

Figure 4. *Effects of Linuron contaminated soil on carbohydrate content (µg/g individual) during different treatment periods. Highly significant difference (p = 0.0001)*

Effect of Linuron on total lipid content

As shown *Table 4* and *Figure 5*, the total lipid content (µg/g individual) differs significantly ($p = 0.0000$) following exposure periods (24, 48, 72 h) between controls and treated earthworm at doses of 250, 500, 1000 µg/500 mg soil. Additionally, the lipid content increased significantly in treated earthworm at doses of 250, and 500 µg compared with the controls after 24 h, but dropped after 48 h of treatment, and kept relative to the control with the three used doses after 72 h of treatment.

Table 4. *Statistical description of lipid content during different treatment periods*

Dose	Mean	Median	Min	Max
24 h				
Control	38.67	17.33	17.33	81.33
D1	50.22	41.00	26.67	83.00
D2	178.33	125.00	90.00	320.00
D3	24.78	26.67	18.67	29.00
48 h				
Control	23.44	29.33	28.67	33.33
D1	30.44	22.33	21.67	26.33
D2	28.55	41.67	33.33	104.33
D3	59.78	21.67	15.00	49.00

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72 h				
Control	178.44	162.33	136.00	219.00
D1	92.22	90.67	54.67	131.33
D2	80.44	73.67	47.00	120.67
D3	96.78	96.33	89.67	104.33

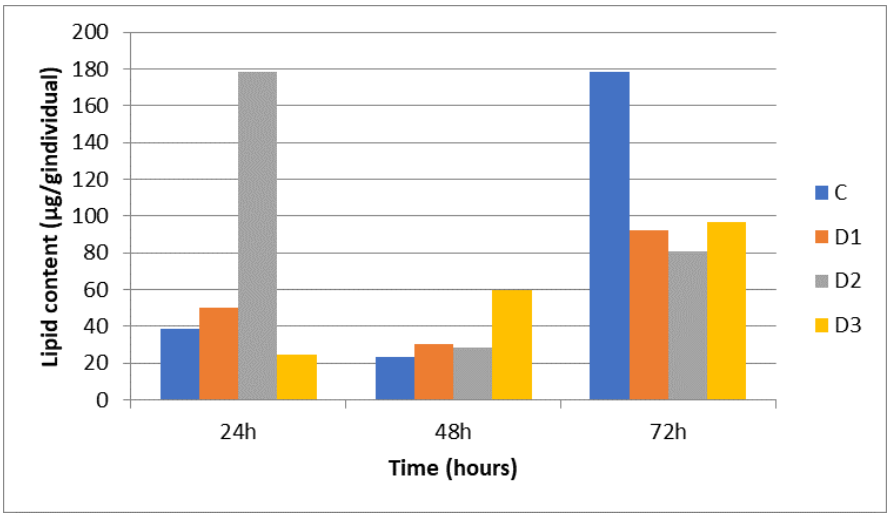

Figure 5. *Effects of Linuron -contaminated soil on lipid content (µg/g individual) during the different treatment periods. Significant difference (p = 0.0000)*

Effect of Linuron on catalase activity

Table 5 and *Figure 6* revealed no significant difference in catalase activity between control earthworm and those treated with Linuron during the three treatment periods (p = 0.1518).

Table 5. *Statistical description of catalase during different treatment periods*

Dose	Mean	Median	Min	Max
24 h				
Control	0.19	0.44	0.15	0.44
D1	0.28	0.10	0.07	0.18
D2	0.35	0.36	0.26	0.43
D3	0.29	0.29	0.07	0.48
48 h				
Control	0.099	0.15	0.14	0.27
D1	0.14	0.10	0.08	0.11
D2	0.19	0.05	0.01	0.82
D3	0.29	0.096	0.09	0.23
72 h				
C	0.19	0.19	0.17	0.22
D1	0.29	0.14	0.11	0.64
D2	0.12	0.13	0.10	0.13
D3	0.097	0.13	0.002	0.16

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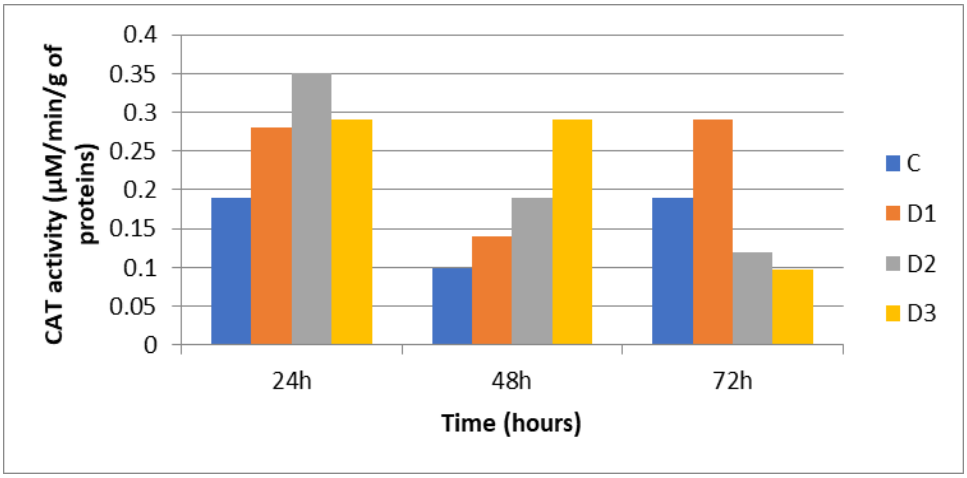

Figure 6. *Effects of Linuron contaminated soil on CAT activity during different treatment periods. Non-significant difference (p = 0.1518)*

Effect of Linuron an biochemical parameters of L. terrestris

The non-parametric Kruskal-Wallis test revealed a non-significant difference between the tested doses of Linuron (250, 500, 1000 µg), and the resulting effect on *L. terrestris* (*Table 6*).

Table 6. *Results of dose–effect of Linuron on the four studied parameters in L. terrstris tested by Kruskal-Walis test (H and p values).*

	Protein		**Carbohydrate**		**Lipid**		**Catalase**	
	H	***P***	***H***	***P***	***H***	***P***	***H***	***P***
24 h	4.59	0.2	7.33	0.57	20.08	0.56	3.41	0.33
48 h	1.92	0.59	5.53	0.14	3.31	0.35	2.7	0.44
72 h	1.66	0.65	1.66	0.65	3.41	0.33	5.2	0.16

Of note, the statistical analysis test was supported by studying the correlation between the metabolic variables (proteins, carbohydrates, lipids, and the CAT activity) in *L. terrestris*. As a result, the total protein and total lipid contents were found to be positively correlated with each other (r = 0.50, r^2 = 50%) (*Table 7*).

Table 7. *Spearman correlation matrix of different metabolic variables dosed in L. terrestris during different treatment periods*

	Proteins	**Lipids**	**Carbohydrates**	**CAT**
Proteins	1.00	**0.49***	-0.11	0.06
Lipids		1.00	0.05	0.07
Carbohydrates			1.00	-0.16
CAT				1.00

*Significant differences between values

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Discussion

Earthworms play crucial roles in soil ecosystem processes, including the viability of agro ecosystems, which can be degraded by intensive farming activities such as pesticide use. Consequently, earthworms are mainly affected by herbicides, causing increased mortality rate and decreased overall biomass, density, fecundity, and body growth, in addition to inducing changes in individual behavior through altering the internal tissues and disruption of enzymatic activities (Zhou et al., 2007; Pelosi et al., 2013). Hence, the biochemical responses of organisms via toxic contaminants are evidenced by marked changes in the major biochemical and enzymatic parameters, enabling the effective diagnosis for assessing the toxicological impacts (Mc Loughlin et al., 2008; Swiatek, 2019). As well reported Fadila et al. (2014) and Gamet (2011), chemicals-induced oxidative stress–mediated reactive oxygen species (ROS) generation causes important alterations in the cellular components, including proteins, lipids, carbohydrates, and nucleic acids.

Moreover, proteins play a key role in the major vital functions in the body and make a very important source of energy, even if they are mainly involved in cell architecture requiring very high protein levels. In this context, our results showed a significant increase in protein levels in *L. terrestris* treated by Linuron over different treatment periods, and this is likely due to the detoxification process. Several recent studies have shown that the increase in protein levels is a physiological indication of the mechanism of adaptation to toxic stress and to the development of an antioxidant defense system against pesticides. Our results are in the same line as those previously reported by Mosleh (2006), showing a significant change in total protein in *Tubifex tubifex* worms treated with cadmium. Similarly, another study of Zeriri et al. (2012) revealed an increase in total protein levels in *Octodrillus complanatus* earthworms treated with Methomyl (Mohamed et al., 2016), as previously reported that the total protein levels increased significantly ($p = 0.003$) in *L. terrestris* treated with the herbicide (Sekator), TSP (triple superphosphate) and the Sekator/TPS mixture, and similarly (Berrouk et al., 2022) reported that the total protein levels increased significantly ($p = 0.0000$) in *L. terrestris* treated by Mikel-Flash (fungicide). However, decreased protein levels were reported by Padjama and Rao (1994) in *Bellammya dissimillis* treated with organophosphate insecticides, and in *Aporrectodea caliginosa* treated with NPK fertilizer (Halaimia et al., 2021). Li et al. (2020) have proven that the herbicide sulfentrazone causes biochemical responses and histological in *Eisenia fetida*. On the other hand, several studies have revealed the importance of plants as bio indicators, such as lichens to monitor air pollution, which, under stress conditions, increased the level of their proteins playing an important role in detoxifying these contaminants by forming complexes (proteins/xenobiotics) (Stalet et al., 2003).

Furthermore, carbohydrates form a very important group of compounds that can be rapidly and immediately used by the body, since they represent a source of energy either in the form of reserves (glycogen), or structural form (cellulose and chitin) (Mouassard, 1999). Accordingly, the obtained results showed a significant difference after treatment with Linuron over the three experimental periods. Mouassard (1999) reported that the increase in carbohydrates is necessary to generate the energy needed to cope with pesticide-induced intoxication. Carpy et al. (2000) has also explained this increase by structural changes in cell membranes and their permeability. Moolman et al. (2007) reported that Carbohydrates have been suggested to be the first energy sources mobilized by living cells under toxic stress. A similar study of Groppa and Benavides

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(2008) revealed that glycogen and lipids, the main energy storage compounds, are the preferred energy fuel offered to tissues in case of cellular needs. In this regard, a highly significant increase in total carbohydrates content was found in *Saccharomyces cerevisiae* yeast after treatment with both 5 mM ($p = 0.004$) and 10 mM ($p = 0.003$) concentrations of cadmium (Fadila et al., 2014). Li et al. (2019), mentioned that *Eisenia fetida* bio accumulates pentachloronitrobenzene, which is explained by variation in oxidative stress parameters. Meanwhile, a significant reduction in carbohydrates was observed in *Aporrectodea caliginosa* earthworms treated with NPK fertilizer (Halaimia et al., 2021). According to Deraissac (1992), the process of total carbohydrate content in the tissues of stressed plants is recognized as a characteristic of environmental adaptation under environmental stress.

Further, lipids are the main components of cell membranes and organelles and an important energy source after carbohydrates, and thus they play an important role in cellular functions. According to Favier (2003), fatty acids are the preferred targets of oxidative stress agents, and noteworthy, the lipid attack involves circulating lipoproteins or membrane phospholipids, resulting in alteration of membrane fluidity and disrupting the function of cell-surface receptors and transporters. Our results showed significant differences in lipids content between control and treated earthworms (Eissa et al., 2002) indicating that the harmful effect of all chemical compounds could be attributed to increased energy utilization (lipid source) or organelle alterations. Significant reductions in lipids content were reported in *Aporrectodea caliginosa* treated with NPK (fertilizer) (Halaimia et al., 2021) and in *Bellammya dissimillis* snails treated with organophosphate insecticides (Padjama and Rao, 1994). It was previously found that iron oxide nanoparticles significantly decreased total lipids content in *Helix aspersa* Sana et al. (2016) and Selvara et al. (2019) reported that a Cd directly affected lipid metabolism and induced the synthesis of lipid droplets in *Saccharomyces cerevisiae.* Sifi and Soltani (2019) found that the environmental pollutants of Sidi Salem region in Annaba city (Northeast Algeria) caused a significantly decreased of the level of total lipids in bivalves *Donax trunculus.*

Endogenous enzymatic antioxidants, including CAT are considered the first line of cellular defense against oxidative damage, preventing the induction of oxidative stress and playing an important role in the elimination of free radicals (Lukyanenka et al., 2013). In this sense, our results showed no significant differences between control and treated earthworm during the different treatment periods. This is likely explained by the non-induction of CAT in the detoxification process possibly because of the short exposure periods or the low used doses. Our results are in good agreement with those previously reported by Hosni and Stenersen (1999), showing no significant differences in CAT in *Eisenia fetidea* earthworms exposed to different concentrations of paraquat. Similarly, Han et al. (2014) reported no variation in CAT activity in *Eisenia fetidea* exposed to azoxytrobin. Givaudan (2014) found that CAT decreased in *Allolobophora chlorotica* which populates soils contaminated by pesticide residues. While catalase activity was increased in *Aporrectodea caliginosa noctura* exposed to chlorpyrifos-ethyl and lambdo-cyhalothrin (insecticides) and myclobutanil and metalaxyl (fongicides) (Schreck et al., 2012). Similarly, Zeriri et al., 2012 reported the increase in the CAT rate in *Octodrillus complanatus* after three days of application of the insecticide. Also Mima and Branimit (2013), showed that pirimiphos-methyl causes an increase in CAT rate in *Eisenia Andrei*. Singh et al. (2019) found that triazophos and deltamethrin and their mixture also cause a remarkable effect on AchE in *Eudrilus*

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eugeniae. According to Yufer et al. (2020), the increasing of the concentrations of Atrazine, causes decrease in CAT levels in soil microorganisms. Belabed and Soltani (2022), mentioned that *Donax trunculus* exposed to Cd showed an increase in CAT level, which will be decreased after their transfer to an uncontaminated medium, which proves the induction of CAT in detoxification and stimulation of antioxidant system. This proves the CAT's induction of detoxification and stimulation of the antioxidant system. On top of that, Spearman's correlation coefficient analysis of the linear relationships between biochemical and enzymatic parameters over the three exposure periods showed that protein content was correlated with lipids content, and this can be explained by the fact that both metabolites are energy reserves and natural products that represent immediately usable energy sources, acting mainly against attacks of harmful pollutants. Herein, a previous study, Sifi and Soltani (2019) reported a negative correlation between protein and carbohydrate contents with MDA content. Last but not least, the biochemical and antioxidant markers are insufficient to discuss the responses of earthworms undergoing stress conditions, and actually, several factors can influence the response of biomarkers, such as the animal's health, age, size, stage of development, and reproductive period, seasons and temperature (Gilis et al., 2002). This is supported by the results of Grey and Richard (1991), showing an increase in the antioxidant system activity following a decrease in chemical stress.

Conclusion

Lumbricus terrestris and all earthworms are bio indicators of biodiversity, as well as bio indicators of soil contamination by various substances, such as pesticides and heavy metals. The aim of our work was undertaken to assess the effect of the herbicide Linuron on several biochemical parameters in a biological model, *L. terrestris*. Biochemical analysis of metabolites content (total proteins, total carbohydrates, and total lipids) in *L. terrestris* earthworms treated with m Linuron at different doses (250 µg, 500 µg, 1000 µg/500 mg soil) and in different exposure periods (24, 48, 72 h) showed marked effects on the defense system in the candidate species, as evidenced by a significant increase in the levels of the three metabolites compared with those of control. Measurement and determination of CAT activity in the same species, with the same doses of the herbicide over the same exposure periods, showed no significant differences between control and treated earthworms, and this is explained by the fact that Linuron does not act on the oxidative stress system.

Conclusively, *L. terrestris* proved as an excellent biological model for use as a bio-indicator of the terrestrial environment pollution by various types of pollutants such as Linuron.

To continue studying the effects of Linuron on *L. terrestris*, it would be advisable to:

- Increase herbicide doses and test them over longer periods (7, 14, and 28 days, four month).
- Assay other enzymatic parameters involved in the metabolism/detoxification process, such as GSH, GST, MDA, AchE and APx.
- Initiate histological and ultrastructural studies.
- Test the effect of Linuron on *L. terrestris* reproduction.
- Test the effect of Linuron on other earthworm species, such as *Aporrectodea giardi, Aporrectodea trapenazoides, Eisenia fetide and Eisenia andrei.*

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REFERENCES

[1] Abdallah, S. M. (2012): Humoral defense response of earthworms *Lumbricus terresteris* against, herbicide; nominee. – J. Plant. Prot and Path, Mansoura Univ 3(9): 881-895.
[2] Bahandri, G., Atrey, K., Scheepers, P. T., Geissen, V. (2020): Concentration and distribution of pesticide residues in soil: non-dietary human health risk assessment. – Chemosphere 253: 126594.
[3] Belabed, B., Soltani, N. (2022): Cadmium Exposure on *Donax trunculus* (Mollusca: Bivalvia) Lethality parameters and Biomarkers Responses. – Fresenius Environmental Bulletin 31(12): 11715-11725.
[4] Berrouk, H., Madjri, S., Hachad, Z., Boukhili, K., Touarfia, M., Kaouachi, N., Khaldi, F. (2022): Effects of Fungicide Fosetyl AL 50% + Folpet 25% (Mikal-Flash) on Biochemical and Histological Aspects of *Lumbricus terrestris* Earthworms. – Environment and Ecology 40(D4): 2842-2850.
[5] Berrouk, H., Guerfi, S., Bendjebbar, R., Bendjaballah, N. E., Laoudja, K., Khaldi, F. (2023): Effect of copper and iron on earthworms *Eudrillus eugeniae* (Kinberg 1967) (Oligochaeta, Eudrilidae) under laboratory conditions. – Environment and Ecology 41(2): 1217-1224.
[6] Bouché, M. B. (1972): Lombriciens de France. Ecologie et systématique. – Ann. Soc. Ecol. Anim. 72: 1671.
[7] Bratford, M. M. (1976): A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principal protein-dye binding. – Anal. Biochem.72: 248-254.
[8] Carpy, S. A., Kobel, W., Doe, J. (2000): Health risk of low-dose pesticides mixture: a review of the 1985-1998, literature on combination toxicology and health risk assessment. – Journal of Toxicology and Environmental Health. Part B: Critical Review 3(1): 1-25.
[9] Claiborne, A. L. (1985): Catalase Activity. – In: Greenwald, R. A. (ed.) CRC Handbook of Methods for Oxygen Radical Research CRC Press, Boca Raton, FL.
[10] Connell, D., Lam, P., Richardson, B., Wu, R. (1999): Introduction to Ecotoxicology. – Blackwell Science. Ltd., Oxford.
[11] Dagnilie, P. (2006): Theoretical Applied Statistics. Description Statistics and Bases of Statistical Inference. Vol. 1. – De Boeck and Larcier, Brussels.
[12] Dagnilie, P. (2007): Theoretical Applied Statistics. Description Statistics and Bases of Statistical Inference in One and Two Dimensions. Vol. 2. – De Boeck and Larcier, Brussels.
[13] Denoyelle, R., Rault, M., Mazzia, C., Maxle, O., Capowiez, Y. (2007): Cholinesterase Activity as biomarker of pesticides exposure in *Allolobophora chlorotica* earthworms living in appele orchards under different management strategies. – Environmental Toxicology Chemistry 26: 2644-2649.
[14] Deraissac, M. (1992): Mécanismes d'adaptation à la sécheresse et maintien de la productivité des plantes cultivées. – Agro Trop. 46: 29-39.
[15] Duchateau, G., Florkin, M. (1959): Sur la Threalosémie des insectes et sa signification [For treahalosemie of insects and its signification]. – . Arch. Int. Physiol. Biochem [Arch. Insect. Physiol. Biochem]. 67: 306-314.
[16] Eissa, S. H., Rizk, E. T., Abou-Shafey, A. E., Mona, M. H., Atlum, A. (2002): Toxicological effect on Euphorbia peplus water suspension on heamocytes of the fresh water snails. – Proc LCBS 2: 417-447.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_31873203

[17] Fadila, K., Nedjoud, G., Amina, B., Imen, T., Khouloud, B. (2014): Evaluation of the effect of oxidative stress generated by Cadmium in the yeast *Saccharomyces cerevisiae*. – Advances in Research 2(3): 119-128.
[18] Favier, A. (2003): Le stress oxydant : Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique [Oxidative stress conceptual interest and experimentation in understanding disease mechanisms and therapeutic potential, biochemical mechanisms]. – L'actualité chimique [Chemical News] 11-12: 108-115.
[19] Gamet, P. L. (2011): Physiological impact of pesticides mixture. – Toxicology 46(2): 82-85.
[20] Gilis, P. L., Diener, L. C., Reynoldson, T. B., Dixon, D. G. (2002): Cadmium induced production of metallothionein –lik protein in *Tubifex tubifex* (oligochaeta) and chirononus riparus (Diptera): Correlation with reproduction and growth. – Environ Toxicol. Chem. 21: 1836-1844.
[21] Givaudan, N. (2014): Stratégies d'adaptation de la biodiversité du sol a la pollution environnementale résiduelle établie en paysage agricole. – Thèse de Doctorat. Université de Rennes 1. France.
[22] Goldswortthy, G. L., Mordue, W., Guthkelch, L. (1972): Studies on insect adipokinectic hormones. – Gem. Comp. Endocrinol 18(3): 545.
[23] Grey, W. W., Richard, D. G. (1991): Prooxidant and antioxidant mechanisms in aquatic organisms. – Aquatic Toxicology 19(2): 137-161.
[24] Groppa, M. D., Benavides, M. P. (2008): Polyamines and abiotic stress: recent advances. – Amino Acids 34: 35-45.
[25] Halaimia, S., Tine, S., Tine, D. F., Soltani, N. (2021): Potential side effects of a fertilizer on growth, biochemical composition and biomarker responses of the grey worm *Apporectodea caliginosa*. – Applied Ecology and Environmental Research 19(2): 1247-1266.
[26] Han, Y., Zhu, L., Wang, J., Xie, H., Zhang, S. (2014): Integrated assessment of oxidative stress and DNA damage in earthworms *Eisenia fetidea* exposed to azoxystrobin. – Ecotoxicol. Environ. Saf 107 214-219.
[27] Hosni, T. G., Stenersen, J. V. (1999): Non-inducibility of antioxidant enzymes in the eartworms *Eisenia veneta* and *Eisenia Andrei* after exposure to heavy metals and parquet. – Pedobiologia 43: 652-657.
[28] Jansirani, D., Nivethitha, S., Singh, M. V. P. **(**2012): Production and utilization of vermicast using organic wastes and its impact on *Trigenella foenum* and *Phaseolus aureus*. – Int J Res Sci. 2(4): 187-189.
[29] Journal officiel de la république Algérienne (1984): Fixant la composition consistance et limites territoriales des communes de la wilaya de Souk-Ahras. – Décrit N°84-365.
[30] Lavelle, P., Spain, A. (2001): Soil Ecology. – Kluwer Scientific Publication, Amesterdam.
[31] Le Bayon, R. C., Amossé, J. D. F., Baros, G., Sandoz, G., Fournier, B., Mitchelle, E. A. D. (2016): Pattrens of earthworm enchytraeid and nematode, diversity and community structure in urban soils of different ages. – Europ J Soil 73: 46-58.
[32] Li, M., Ma, X., Saleem, M., Wang, X., Sun, I., Yang, Y., Zhang, Q (2020): Biochemical response, histopathological change and DNA damage in earthworm (*Eisenia fetida*) exposed to sulfentrazone herbicide. – Ecological Indicators 115(10): 64-65.
[33] Li, M., Xu, G., Yu, R., Wang, Y., Yu, Y. (2019): Bioaccumulation and toxicity of pentachloronitrobenzene to earthworm (*Eisenia fetida*). – Ecotoxicology and Environmental Safety 17(4): 429-434.
[34] Lordache, M., Borza, I. (2011): Study of the acute toxicity of some pesticides on earthworms *Eisenia fetidea*. – Res. J. Agric. Sci. 43(4): 95-100.
[35] Lukyanenka, L. M., Skarabahatava, A. S., Slobozhanina, E. L., Kovaliova, S. A., Falcioni, H. L. (2013): In vitro effect of AICL3 on humanerythrocytes changes in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_31873203

membrane morphology and functionality. – Journal of Trace Elements in Medicine and Biology 27:160-167.
[36] Maksyniv, I. (2015): Pesticides benefits and hazards. – Vasyl. Stefanyk. Precarpathian National University 2(1): 70-76.
[37] Martin, F. L., Martinez, E. Z., Stopper, H., Garcia, S. B., Uyemura, S. A., Kannen, V. (2018): Increased exposure to pesticides and colon cancer; early evidence in Brazil. – Chemosphere 209: 623-631.
[38] Mc Loughlin, N., Yin, D., Malthy, I., Wood, R. M., Yu, H. (2008): Evaluation of sensitivity and specificity of two crustacean biochemical biomarker. – Environment. Toxicology. Chemistry.19: 2085-2092.
[39] Mima, V., Branimir, K. H. (2013): Biomarker responses in earthworm *Eisenia Andrei* exposed to pirimiphos methyl and deltamethrin using different toxicity tests. – Chemosphere 90(3).
[40] Mohamed, N. M., Samir, T., Taha, M., Hind, A., Hafsia, S. (2016): In vitro Biomarker Responses of eartworm *Lumbricus terresteris* exposed to herbicide Sekator and phosphate fertilizer. – Water, Air and Soil Pollution 277: 15.
[41] Moolman, I., VanVuren, J. H. J., Wepener, V. (2007): Comparative studies on the uptake and effects of cadmium and zinc on the cellular energy allocation of two freshwater gastropods. – Ecotox Environ Safe 68: 443-450.
[42] Mosleh, Y. Y., Paris, P. S., Biagianti, R. S. (2006): Metallothioneins induction and antioxidative response in Aquatic worms *Tubifex tubifex*, exposed to copper. – Chemosphere 64(1): 121-128.
[43] Mouassard, C. (1999): Structural and Metabolic Biochemistry. – Medicine, Pharmacy, Sciences de Boeck, Brussels.
[44] Multigner, L. (2005): Delayed effects of pesticides on human health. – Environnement Risques Santé 4(3): 187-194.
[45] Padmaja, R. J., Rao, M. B. (1994): Effect of an organochlorine and three organophosphate pesticides on glucose, glucogen, lipid and protein contents in tissues of the freshwater snail *Bellamya dissimilis* (moiler). – Bull. Environ. Contam. Toxicol. 53: 142-148.
[46] Pelosi, C., Sebastian, B., Yvan, C., Mickael, H., Franck, V. (2013): Pesticides and Earthworms. – Agro Sustain Dev. pp 1-33.
[47] Pretty, J and Pervez, B. Z. (2015): Integrated pest management for sustainable intensification of agriculture in Asia and Africa. – Insects 6(1): 152-182.
[48] Redriguez-Eugenio, N., Mc Laughlin, M., Pennock, D. (2018): Soil Pollution: A Hidden Reality. – Land and Water Development Division, Food and Agriculture Organization (FAO), Geneva.
[49] Reinecke, S. A., Reinecke, A. J. (2007): The impact of organophosphate pesticides in orchards on earthworms in the western Cap. South Africa. – Ecotoxicology and Environmental Safety 66: 244-252.
[50] Sana, B., Samira**,** B., Labiba, Z., Charaira, S., Smina, A. H., Houria, B. (2016): Impact of nanometric iron oxide in the hepatopancreas of terrestrial gasteropod *Helix aspersa*, Histological changes and Biochemical parameters. – Int. J. Pharm, Sci. Rev. Res. 37: 234-241.
[51] Scherrer, B. (2007): Biostatistic. Vol. 1. – Gaëtan Morin, Boucherville.
[52] Schreck, E., Foucault, Y., Sarret, G., Sobanska, S., Cecillon, L., Castrec, R. M. (2012): Metal and Metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: mechanisms involved for lead. – Science of the Total Environment 427-428: 253-262.
[53] Selvara, J. R., Albert, A., Govindan, S. S., Vasanthi, N. (2019): Effect of cadmium on essential metals and their impact on lipid metabolism in *Saccharomyces cerevisiae.* – Cell Stress and Chaperones 25: 19-33.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_31873203

[54] Shibko, S., Koivistoinen, P. T., Tratyneek, C., Newhall, A., Freidman, L. (1966): A method of the sequential quantitative separation and glycogen from single rate liver homogenate on from sub-cellular fraction. – Anal. Biochem 19: 415-428.
[55] Sifi, K., Soltani, N. (2019): Seasonal changes of two biomarkers of oxidative stress (LDH, MDA) in the edible Mollusk *Donax trunculus* from the golf of Annaba (Algeria), correlation with carbohydrate and lipid contents. – Molluscan Research 39(1): 44-52.
[56] Singh, S., Tiwari, B. K., Pandy, R. S. (2019): Acute toxicity evaluation of triazophos, deltamethrin and their combination on earthworm, *Eudrilus eugeniae* and its impact on AChE activity. – Chemistry and Ecology 35(6): 563-575.
[57] Smolders, R., Baillien, I. M., Blust, R. (2005): Relationship between the energy status of Daphnia magna and its sensitivity to environmental stress. – Aquatic. Toxicol 73: 155-170.
[58] Stalet, J. P., Sneller, F. E., C., Bryngelson, T., Lumdborg, T., Schat, H. (2003): Phytochelatin and Cadmium accumulation in Wheat. – Environmental and Experimental Botany 49: 21-28.
[59] Swiatek, M., Bednarska, A. J. (2019): Energy reserves and respiration rate in the earthworm *Eisenia andrei* after exposure to zinc nanoparticule or ionic Zuzanna. – Environ. Sci. Poll. Res. 26: 24933-24945.
[60] Yufei, L., Xiaoxu, F., Tong, Z., Wenyuan, H., Fuqinag, S. (2020): Effects of the long term application of Atrazine on soil enzyme activity and bacterial community structure in farmlands in China. – Environmental Pollution 262: 114264.
[61] Zeriri, I., Tadjine, A., Grara, N., Belhouchet, N., Berrebbah, H., Djeber, M. R. (2012): Potential toxicity of an insecticide of the family of carbamates on bio indicator model of the pollution the earthworm *Octodrillus complanatus*. – Annals of Biological Research 5367-5373.
[62] Zhou, S. P., Duan, C. P., Fu, H., Chen, Y. H., Wang, X. H., Yu, Z. E. (2007): Toxicity assessment for chlorpyrifos contaminated soil with three different earthworm test methods. – Journal of Environmental Sciences 19(7): 854-858.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_31873203