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# Impacts of Short-term Exposure of Caselio (plant fertilizer) On The Freshwater Snail, *Lanistes carinatus* (Ampullariidae, Mollusca).

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**ABSTRACT**

Ecotoxicological effects of agriculture products especially fertilizers on aquatic invertebrates is a crucial case to study. The effect of Caselio, the fertilizer (Plant Metabolism Regulators, PMR) on aquatic organisms' response is of great importance. The freshwater snail, *Lanistes carinatus* was collected from Bahr Shebeen canal, Menoufia Province, Egypt. Snails were exposed to 200 and 600 µl/l of the plant fertilizer (PMR) for 0, 1, 3 and 7 days. Endpoints investigated were $LC_{50, 90}$, condition index (CI), shell, operculum, foot and soft tissue metals concentrations (Zn, Fe, Mn and Ca), metallothioneins (MTs) and antioxidants (Catalase; CAT and Total Glutathione; GSH) of gonad-digestive complex. $LC_{50}$ and $LC_{90}$ values were 1.3 and 4.2 ml/l, respectively. CI of the exposed snails reduced significantly especially on the 1$^{st}$ day (17.5 % reduction). The plant fertilizer (PMR) exposure caused metal accumulation mainly in digestive-gonad complex, followed by the shell, foot and lastly the operculum. Exposure to the plant fertilizer (PMR) resulted in lower Ca concentrations in shell, but disturbed/increased its content in the rest tissues of the exposed snails (ANOVA/Kruskal Wallis, $P \leq 0.05$). Metallothioneins increased 1.5 fold in the plant fertilizer (PMR) exposed snails` digestive-gonad complex (200 and 600 µl/l) when compared to the control group (3223.2 ± 127.5, 2797.6 ± 119.4 and 2076.4 ± 73.8 pg/mg, respectively). The plant fertilizer (PMR) caused increase in the activity of the antioxidants CAT but decrease in GSH concentration significantly (Kruskal Wallis, $P \leq 0.05$) in the digestive-gonad complex of the exposed snails. It can be conclude that the plant fertilizer (PMR) exposure affected the biological responses in *L. carinatus* with specific tissue response and caused oxidative stress. Digestive-gonad complex, foot and shell could be used as target organs and biomarkers for metals accumulation; however, operculum was not a target organ for metal accumulation.

**Keywords:** Fertilizer, *Lanistes carinatus*, Metals, Metallothioneins, Antioxidants.

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## INTRODUCTION

Molluscs have several advantages for using them as an animal model for their enough tissue mass, almost sedentary lives and easy to identify [1]. More than 35,000 living species are gastropods, mainly snails [2]. Genus *Lanistes* belongs to Family Ampullariidae and distributed in the old world at Sudan, Ethiopia, Uganda, Kenya and Somalia [3] and Yemen [4] and in Egypt at the river Nile and its canals [5]. The freshwater snails were usually used as bioindicators for aquatic metal pollution [6]. Freshwater gastropods can be used for the biological control of intermediate hosts of some diseases. *Lanistes carinatus* was examined for consumption of *Biomphalaria glabrata* egg masses and juveniles, proved its effectiveness for *B. glabrata* control [7], and affected the growth and populations of *B. glabrata* [8].

Freshwater ecosystems located in vicinity of agricultural landscapes are exposed to continuous contamination by agricultural drains (ex. pesticides, fertilizers, insecticides and herbicides) through various transport routes. These routes could be spray drift, surface or underground runoff, drainage or wet or dry deposition [9, 10, 11, 12]. Fertilizers are any material of natural or synthetic origin that was applied to soil or to plant tissues to supply the plant with nutrients essential to its growth. The plant fertilizer is composed of macro- and micronutrients. The main macronutrients are nitrogen (N), phosphorus (P), and potassium (K), but not in their elemental form. Generally, they are good for development of different parts of the plant or for movement of water in them. Sometimes, micronutrients like essential metals (Fe, Zn and Mn) were added to the fertilizers to regulate the plant metabolism [13]. Urea fertilizers were used for aquatic plants productivity, duckweed plant [14]. Fertilizer' effects on the aquatic biota have been discussed in several studies. Gudleifsson [15] recorded decrease in the number of invertebrate species and soil fauna but replaced by increase in the number of mites and springtails after use of fertilizers. Spraying the common fertilizer, sodium ammonium nitrate caused massive reduction of insects which eaten by the Loggerhead Shrikes bird *(Lanius ludovicianus)* and caused mortality to their eggs and nestlings [16]. The mineral N fertilizer increased the microbial biomass 15% of the soil more than the unfertilized control, which associated with change in pH [17].

The accumulation of metals in specific organs of marine bivalves has been proposed as a more sensitive and specific indicator of water contamination than whole body burdens [18, 19, 20]. Alteration in the calcium content is important for the cells and shell of molluscs and hyper/hypocalcification induced in the tissue and shell was discussed [21]. Calcium plays an important role in the lives of molluscs [22] and plays a vital role in the nerve–muscle transmission [23]. Moreover, high concentrations of calcium chloride reduced the reproduction of the freshwater snails, *Planorbarius corneus* [24]. In addition, calcium concentration in water affected the distribution of molluscs in aquatic system, as total species were significantly correlated with calcium [25].

Metallothioneins (MTs) are low molecular weight non-enzymatic proteins with high affinity to metals. They act for homeostasis of the essential metals (Zn, Cu and Fe), and detoxification of non-essential metals (Hg, Cd, and Ag) in nearly all organisms (animals, plants and bacteria). They were expressed also under the condition of oxidative stress [26, 27, 28]. During the exposure of *Talitrus saltator* (crustacean) to Cu, the MTs content increased until 24 h in groups exposed to 5 ppm and throughout the duration of the experiment in amphipods exposed to 10 ppm [29]. Rainbow et al. [30] discussed the different categories of accumulated trace metals. They mentioned it was a process of subcellular fractionation and depended on the subcellular component to which metal is bound.

Antioxidants are divided into enzymatic and non enzymatic defences. The first one is like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The second one is tocopherol (Vitamin E), ascorbic acid (Vitamin C), glutathione (GSH), flavonoids and carotenoids. Under normal conditions, there is a balance between the intracellular levels of these antioxidants. This balance is essential for the health of the cells [31]. The oxyradicals generation produces biological damage depends on the effectiveness of antioxidant defences [32]. Catalase plays an important role in catalyzing the decomposition of hydrogen peroxide to water and oxygen [33]. GSH is involved in scavenging free radicals known to block the spreading of lipid peroxidation and a substrate for both GST and GPx [34, 35]. Snails, *Theba pisana* collected from polluted sites with urban heavy metal exhibited increased in CAT and decreased in GSH than snails from reference site at Alexandia, Egypt [36]. Ferreira and Alencastro, [37] recorded 31% drop in catalase activity after exposure of the freshwater snail, *B. tenagophila* to anoxia.

The aim of the present study was to determine the adverse effects of Caselio, the plant fertilizer (PMR) on the freshwater snail, *Lanistes carinatus* and in turn use it as a biomonitor for pollution caused by agricultural products. This will be accomplished by measuring tissue metallothionies and antioxidants with a special reference to organ-specific accumulation of metals.

## MATERIALS AND METHODS

### Animals

One hundred and fifty snails, *L. carinatus* were collected from Bahr Shebeen Canal at Winter (December, 2014), (total body weight, 7.26 ± 0.11 gm) and were transported in field water to the laboratory. Snails were held after collection for two weeks acclimation period in filtered declorinated water in tanks (5 L tanks, length: 25, width: 16, height: 15.5 cm) filled with 1 litre/tank. The snails were maintained under laboratory conditions with photoperiod 12 light: 12 dark and water pH was 7.1 ± 0.4.

### Experimental material

Caselio is a plant metabolism regulator (PMR) in solution form [contains 7% N, 20% $P_2O_3$, 4% Zn, 1.8% Fe, 1.3% Mn and 4.5% free amino acids (w/v)]. A product of international Egypt Chem, is under registry licence No. 2593.

### Determination of $LC_{50}$ and $LC_{90}$ of the plant fertilizer (PMR)

Snails were exposed to a series of concentrations of the plant fertilizer, PMR for 120 h at room temperature (20 ± 2 °C). Triplicates of tanks (10 snails/concentration) were kept under the same laboratory conditions in dechlorinated tap water [38]. Dead snails were removed and recorded from each tank.

### Experimental design

After 2-weeks of acclimatization to the laboratory conditions, adult *L. carinatus* snails were divided into three groups (one control and two concentrations of the plant fertilizer [PMR]). Each group consisted of three replicates (10 individual/replicate). The 1$^{st}$ group (control) was kept in filtered declorinated tap water, the 2$^{nd}$ group treated with the fertilizer, PMR (200 µl/l, $LC_{10}$), and the 3$^{rd}$ group treated with the fertilizer, PMR (600 µl/l, $LC_{30}$). Tanks water were re-dosed every other day after a 100% water change, and snails were fed on fresh green lettuces. Snails were sampled on day 0, 1, 3, and 7 as 3 samples from each group. The assessed parameters were condition index, heavy metals in shell, operculum, foot, and soft tissue, metallothionins, antioxidants in gonad-digestive complex.

### Condition index

The condition index (CI) is an index of current organism nutritive status and a measure of the relative proportions of flesh to shell weight, and was calculated according to Aguirre [39] from the following formula:

$$CI = [MW/ (TW- SW)] \times 100$$

where MW; wet meat (flesh) weight, TW; total wet weight and SW; shell weight.

### Metal analysis in tissue

Tissue samples were prepared according to Federici et al. [40] with minor modifications. Briefly, about 0.05 g of fresh tissue (shell, operculum, foot, soft tissue and gonad-digestive complex) was oven dried, digested in concentrated $HNO_3$ at 70 °C, and then diluted to a final volume of 5 ml with distilled water. Total Zn, Mn, Fe and Ca were measured using Inductively Coupled Argon Plasma, iCAP 6500 Duo (Thermo Scientific, England) against 1000 mg/l multi-element certified standard solution (Merck, Germany) as stock solution for instrument standardization.

**Metallothionens**

Metallothioneins was measured using ELISA kit (SEB119 Mu) according to [41] with minor modifications. Breifly, 0.03 g of snails` gonad-digestive complex of control and treated snails were homogenized with 200 µl of phosphate buffered saline (PBS). Samples were centrifuged at 5,000 g for 5 minutes to collect the supernatant. Chemicals were obtained from Cloud-Clone Corp, USA. The optical density (OD) was measured at 450 nm using the spectrophotometer.

**Antioxidants assays**

**Catalase (CAT)**

Snails` gonad-digestive complex (0.03 g) was homogenized in 200 µl cold phosphate buffered saline (PBS), centrifuged at 4,000 rpm for 15 minutes at 4 °C. The supernatant was collected for the assay. CAT activity was determined according to Aebi [42] using Catalase Assay kit (CA2517, Biodiagnostic, UK). One unit of catalase was expressed by the amount of catalase decomposes 1 µM of $H_2O_2$ per minutes at 25 °C and pH 7 and was expressed as U/g. Optical density (OD) was measured at 500 nm.

**Total Glutathione (GSH)**

Snails` gonad-digestive complex (0.03 g) was homogenized in 300 µl cold metaphosphoric Acid (MPA), centrifuged at 12,000 rpm for 15 minutes at 4 °C. Total Glutathione concentration in snails` gonad-digestive complex was determined according to Mytilineou et al. [43] using OxiSelect™ Total Glutathione kit (STA-312) from Cell Biolabs, INC, USA. Total Glutathione concentration was expressed as µM/mg and the optical density (OD) was measured at 405 nm.

**Statistical analysis**

All data were analysed using Statgraphics (v5.1 software). Data were expressed as mean ± S.E. LC values were calculated using the plot of fitted model of simple regression. The statistical analysis was carried by One-way ANOVA to set the difference between the control and treated groups of the experiment, setting the probability level to $P \leq 0.05$, where ANOVA could not be applied, Kruskal Wallis test was used.

## RESULTS

***Molluscicidal effect of the plant fertilizer (PMR) on adult L. carinatus***

Molluscicidal activity of the plant fertilizer, PMR against adult *L. carinatus* snails after 120 h of exposure was measured. The results showed that $LC_{50}$ and $LC_{90}$ values were 1.3 and 4.2 ml/l, respectively with slope value 1.5 (Fig. 1). The control groups recorded no mortalities (0 %) during the experimental period. On $6^{th}$ day, 600 µl/l of the fertilizer caused mortality of two snails (6.6 %), and 200 µl/l caused death to only one snail (3.3 %) on the $7^{th}$ day of the experiment. Mucous secretion of snails in the treated water was the most obvious observation of the treated snails. Relaxed foot muscle was characteristic of died snails.

***Effect of the plant fertilizer (PMR) on adult L. carinatus CI***

CI was measured at 0, 1, 3 and 7 days of the experiment. No significance was found between the total weight of the control and exposed groups during the experimental period (ANOVA, $P \geq 0.7$). At time zero, CI was 64 ± 4.6. At 1 day, CI of 600 µl/l exposed snails was significantly less than the control group (approx. 17.5 % reduction, ANOVA, $P = 0.05$). For the rest of the time points, CI lost any significant difference between treatments and control groups or between the treated groups and time (ANOVA, $P > 0.05$). Values at the end of experiment on day 7 were 61.4 ± 3.4, 60.7 ± 6.9 and 57.3 ± 4.9, for control, 200 and 600 µl/l treated sanils`, respectively (Fig. 2).

**Effect of the plant fertilizer (PMR) on metal accumulation in *Lanistes carinatus* tissues**

**The shell**

Exposure to the fertilizer, PMR was confirmed by clear evidence of Zn, Mn and Fe accumulation in all the analysed tissues of the snail by day 7. The fertilizer, PMR exposure caused large (1.2 and 2.4 fold) increases in Zn concentration of the shell in the exposed snails to 200 and 600 µl/l, respectively (ANOVA, $P \leq 0.05$, Table 1). Mn concentration increased in 200 µl/l exposed snails shell more than 600 µl/l at 1st and 7th days. Mn increased in exposed snails than the control snails significantly (ANOVA/Kruskal wallis, $P \leq 0.03$). The fertilizer, PMR exposure caused reduction in accumulation of Fe of 600 µl/l exposed snails` shell but increased the accumulation in 200 µl/l exposed snails shell (ANOVA, $P = 0.007$). Ca content in the shell of exposed snails did not follow a pattern in accumulation, but recorded disturbance (increase/decrease) than the control group.

**The operculum**

The fertilizer, PMR exposure caused significant increases in Zn concentration of the operculum of the exposed snails to both concentrations, especially 600 µl/l (Kruskal wallis, $P \leq 0.05$, Table 2). Mn concentration in the operculum of exposed snails recorded no significant difference than the control group during the experimental period except on the 7th day. The fertilizer, PMR (600 µl/l) caused significant increase in Mn concentration on day 7 (ANOVA, $P = 0.003$) more than the control and 200 µl/l exposed groups. Fe content generally recorded significant increase in all exposed snails` operculum (Kruskal wallis, $P \leq 0.05$). Ca content in the operculum of exposed snails` increased in 600 µl/l and decreased in 200 µl/l groups (ANOVA/kruskal wallis, $P \leq 0.05$) than the control group on 1st and 3rd days. However, on the 7th day, Ca content increased in both treatments significantly (ANOVA, $P = 0.03$).

**The foot**

The fertilizer, PMR exposure caused significant increases in Zn concentration of the foot in the exposed snails to both concentrations, especially 600 µl/l treatment (ANOVA, $P \leq 0.05$, Table 3). Mn concentration recorded marginal and non significant increase in both exposed snails` foot than the control one during the experiment. The fertilizer, PMR exposure caused increase in accumulation of Fe for both concentrations at the 1st day. However, slightly decreased or did not affect when compared to the control group for the following 3rd and 7th days. Ca content in the foot of both exposed snail groups increased at the 1st day, and then the increment faded in 600 µl/l treatment. At the 3rd and 7th days, 200 µl/l exposure decreased Ca concentration in the foot than the control group (Table 3).

**The soft tissue**

Zn concentration of the soft tissue of the exposed snails to both concentrations, increased significantly (ANOVA, $P \leq 0.03$, Table 4). Mn content of soft tissue in 600 µl/l exposed snails` recorded significant increase (ANOVA, $P = 0.05$) on 3rd day than 200 µl/l exposed snails. In addition, there was large increase in soft tissue Mn content of exposed snails when compared to the control snails. Fe content generally recorded increase in all exposed snails` soft tissue. In addition, the increment was significant in the 1st day of exposure to both concentrations (Kruskal wallis, $P = 0.02$). Soft tissue content of Ca recorded increase in both exposure groups than the control group on 1st and 3rd days. On the other hand, on the 7th day, Ca content decreased in both treatments but not significantly.

Generally, the fertilizer, PMR caused increase in the accumulation of Zn, Mn and Fe in the exposed snails` shell, foot and soft tissue However, the treatment with the fertilizer, PMR caused disordered accumulation in Ca content of all examined tissues.

Zn mainly accumulated in soft tissue > foot > operculum > shell. For Fe content, it mainly accumulated in soft tissue > operculum > foot > shell. For Mn, soft tissue recorded the highest accumulation followed by operculum > shell > foot. Ca accumulated mainly in the shell followed by the operculum then the soft tissue and finally the foot.

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**Effect of the plant fertilizer (PMR) on adult *L. carinatus* metallothioniens**

Metallothioniens were measured in the digestive-gonad complex at zero, 1, 3 and 7 days. The concentration of metallothioneins at zero time was 2184.1 ± 145.5 pg/mg. Metallothioniens were significantly increased in the exposed snails to both concentrations (200 and 600 µl/l, ANOVA/Kruskal Wallis, $P < 0.05$) at all time points of the experiment when compared to the control group. Meanwhile, the increase in metallothioniens concentration in snails caused by 200 µl/l concentration was higher than by 600 µl/l concentration (1.5 and 1.3 folds, respectively) than the control group at all time points (Fig. 3).

**Effect of the plant fertilizer (PMR) on adult *L. carinatus* antioxidants**

Catalase activity was measured in the digestive-gonad complex at zero, 1, 3 and 7 days (Fig. 4a). Catalase activity was 30.3 ± 2.7 U/g at time zero. Catalase activity was significantly increased in the exposed snails to 600 µl/l (Kruskal Wallis, $P \leq 0.05$) at all time points of the experiment when compared to the control and 200 µl/l exposed groups. However, 200 µl/l increased catalase activity than the control group at all time points but not significantly.

Total glutathione concentration was measured in the digestive-gonad complex at zero, 1, 3 and 7 days. Total glutathione concentration was 30.1 ± 1.2 µM/mg at time zero (Fig. 4b). The concentration of total glutathione was significantly decreased in the exposed snails to both concentrations (Kruskal Wallis, $P \leq 0.03$) at all time points of the experiment when compared to the control group. Exposure to 600 µl/l caused reduction in total glutathione concentration by 40 %, however, 200 µl/l that caused 30 % reduction in its concentration than the control during the experiment.

**Table 1: Total Zn, Mn, Fe and Ca concentrations in the shell of *L. carinatus* for 7 days exposure to the fertilizer (PMR)**

| Time | Treatments | Zn | Mn | Fe | Ca |
|---|---|---|---|---|---|
| Zero | Control | 25.3 ± 5.1 | 50.9 ± 16.2 | 61.9 ± 19.1 | 410896.5 ± 5508.5 |
| 1 day | Control | 26.7 ± 4.3 | 64.9 ± 2.7 | 84.2 ± 7.4 | 413710.9 ± 7143.3 |
| | 200 µl/l | 33.1 ± 5.6 | 182.6 ± 21.6* | 84.7 ± 13.1 | 212586.5 ± 86725.6 |
| | 600 µl/l | 43.8 ± 8.7 | 169.4 ± 27.2* | 68.8 ± 2.4 | 431089.5 ± 53775.9 |
| 3 days | Control | 38.7 ± 5.6 | 87.1 ± 11.4 | 39.8 ± 11.1 | 411832.7 ± 7405.7 |
| | 200 µl/l | 38.7 ± 3.2 | 93.3 ± 6.7 | 147.6 ± 25.6*# | 216878.9 ± 83814.6 |
| | 600 µl/l | 80.4 ± 16.0*# | 133.6 ± 16.2 | 50.1 ± 9.2 | 297667.9 ± 49792.6* |
| 7 days | Control | 39.9 ± 4.4 | 56.1 ± 9.0 | 68.1 ± 10.3 | 415360.1 ± 7561.4 |
| | 200 µl/l | 55.1 ± 12.7 | 252.5 ± 26.5* | 110.2 ± 9.3* | 432158.2 ± 6365.7 |
| | 600 µl/l | 141.9 ± 35.7*# | 190.1 ± 12.7* | 87.7 ± 1.0 | 407990.3 ± 3708.6 |

Note, Data are means ± S.E., in mg/g dry weight (dw), $n = 3$ snails per group. * indicates a significant difference from the control, # indicates a significant difference between treatments (ANOVA/Kruskal-Wallis, $P \leq 0.05$).

**Table 2: Total Zn, Mn, Fe and Ca concentrations in the operculum of *L. carinatus* for 7 days exposure to the fertilizer (PMR)**

| Time | Treatments | Zn | Mn | Fe | Ca |
|---|---|---|---|---|---|
| Zero | Control | 291.8 ± 132.5 | 130.0 ± 42.5 | 395.5 ± 142.8 | 4877.9 ± 1303.0 |
| 1 day | Control | 274.6 ± 36.7 | 133.6 ± 18.4 | 372.2 ± 47.4 | 4247.5 ± 669.6 |
| | 200 µl/l | 576.5 ± 81.9* | 117.5 ± 12.3 | 577.1 ± 88.6 | 3336.8 ± 336.6 |
| | 600 µl/l | 407.5 ± 24.0 | 120.9 ± 4.3 | 436.2 ± 28.4 | 8221.1 ± 516.3*# |
| 3 days | Control | 281.8 ± 35.2 | 128.3 ± 29.2 | 337.6 ± 32.9 | 3071.1 ± 174.1 |
| | 200 µl/l | 256.4 ± 17.2 | 125.0 ± 22.9 | 185.9 ± 47.3 | 1590.1 ± 282.9 |
| | 600 µl/l | 811.6 ± 130.0*# | 135.0 ± 29.0 | 633.3 ± 136.6# | 4718.3 ± 1016.0# |
| 7 days | Control | 327.1 ± 28.0 | 123.3 ± 14.8 | 403.4 ± 22.8 | 2704.3 ± 284.8 |
| | 200 µl/l | 931.9 ± 105.8* | 169.9 ± 27.1# | 646.9 ± 101.7* | 4674.9 ± 585.7* |
| | 600 µl/l | 1317.0 ± 44.8*# | 279.9 ± 9.1* | 470.5 ± 11.6 | 4984.8 ± 526.7* |

Note, Data are means ± S.E., in mg/g dry weight (dw), $n = 3$ snails per group. * indicates a significant difference from the control, # indicates a significant difference between treatments (Kruskal-Wallis, $P \leq 0.05$).

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**Table 3: Total Zn, Mn, Fe and Ca concentrations in the foot muscle of *L. carinatus* for 7 days exposure to (PMR)**

| Time | Treatments | Zn | Mn | Fe | Ca |
|---|---|---|---|---|---|
| Zero | Control | 50.5 ± 5.9 | 13.3 ± 1.4 | 173.7 ± 30.1 | 38545.1 ± 8731.3 |
| 1 day | Control | 55.4 ± 5.9 | 18.0 ± 2.0 | 210.9 ± 47.2 | 24069.2 ± 3597.8 |
| | 200 µl/l | 69.9 ± 12.4 | 24.3 ± 3.5 | 288.8 ± 43.5 | 31268.9 ± 11303.9 |
| | 600 µl/l | 102.3 ± 13.4* | 39.6 ± 11.8 | 347.4 ± 31.4 | 51815.8 ± 9297.5 |
| 3 days | Control | 48.9 ± 13.0 | 17.5 ± 5.2 | 208.8 ± 39.3 | 23452.6 ± 877.5 |
| | 200 µl/l | 41.7 ± 4.7 | 12.3 ± 3.2 | 156.5 ± 25.8 | 15993.4 ± 2424.8 |
| | 600 µl/l | 99.1 ± 14.2*# | 27.3 ± 5.6 | 204.3 ± 44.7 | 28564.6 ± 2665.9# |
| 7 days | Control | 68.4 ± 4.8 | 24.1 ± 4.4 | 209.3 ± 50.0 | 28103.2 ± 2143.7 |
| | 200 µl/l | 65.8 ± 3.1 | 26.9 ± 4.6 | 220.4 ± 38.6 | 17755.7 ± 2559.4 |
| | 600 µl/l | 80.9 ± 1.5*# | 33.1 ± 7.4 | 229.8 ± 14.4 | 21345.3 ± 3288.1 |

Note, Data are means ± S.E., in mg/g dry weight (dw), *n* = 3 snails per group. * indicates a significant difference from the control, # indicates a significant difference between treatments (Kruskal-Wallis, $P \leq 0.05$).

**Table 4: Total Zn, Mn, Fe and Ca concentrations in the soft tissue of *L. carinatus* for 7 days exposure to the fertilizer (PMR)**

| Time | Treatments | Zn | Mn | Fe | Ca |
|---|---|---|---|---|---|
| Zero | Control | 1006.0 ± 242.3 | 753.5 ± 112.7 | 1219.2 ± 294.2 | 26590.3 ± 7675.7 |
| 1 day | Control | 17433.3 ± 391.5 | 962.2 ± 32.7 | 1939.2 ±112.6 | 21854.8 ± 2000.6 |
| | 200 µl/l | 2091.1 ± 417.1 | 2880.7 ± 451.6 | 3803.1 ± 131.3* | 24675.8 ± 182.2 |
| | 600 µl/l | 4032.5 ± 107.2*# | 4051.4 ± 1605.9 | 5024.7 ± 513.4*# | 32419.0 ± 8181.3 |
| 3 days | Control | 1016.5 ± 273.8 | 702.3 ± 113.7 | 1581.2 ± 128.8 | 12219.8 ± 1317.6 |
| | 200 µl/l | 1883.3 ± 144.7* | 1004.5 ± 479.7 | 2774.2 ± 671.9 | 12887.0 ± 1305.4 |
| | 600 µl/l | 2060.9 ± 235.2* | 1354.6 ± 309.8# | 2127.7 ± 40.8 | 15121.6 ± 5176.5 |
| 7 days | Control | 1816.7 ± 156.6 | 803.1 ± 54.8 | 1466.3 ± 302.8 | 19653.1 ± 3656.1 |
| | 200 µl/l | 2034.6 ± 655.0 | 2569.2 ± 715.3 | 3279.6 ± 803.2 | 16964.6 ± 3643.9 |
| | 600 µl/l | 2420.8 ± 150.0 | 3093.9 ± 659.5 | 3657.3 ± 1208.7 | 12422.2 ± 971.8 |

Note, Data are means ± S.E., in mg/g dry weight (dw), *n* = 3 snails per group. * indicates a significant difference from the control, # indicates a significant difference between treatments (Kruskal-Wallis, $P \leq 0.05$).

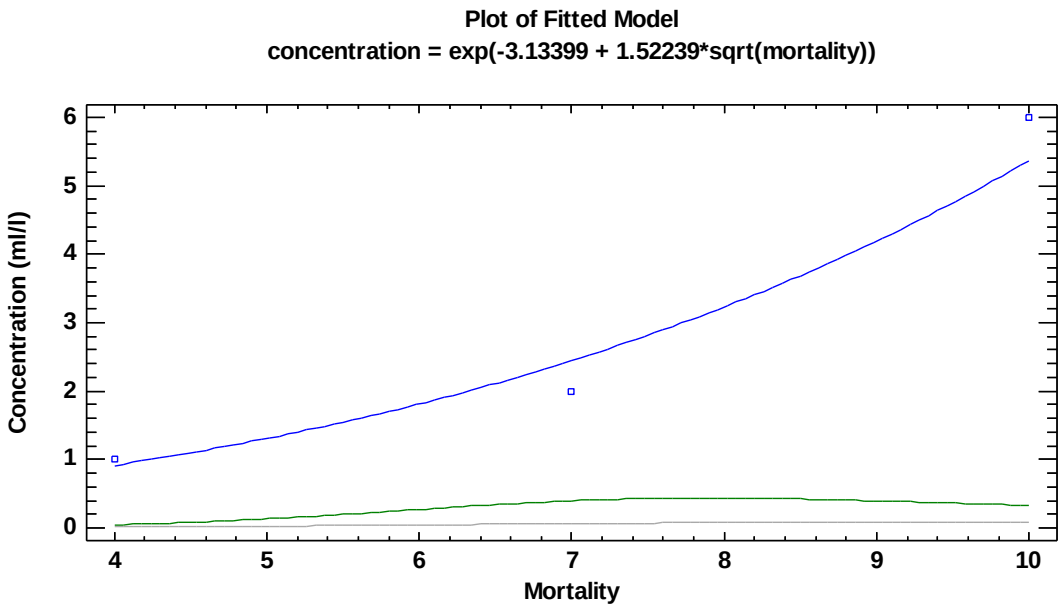


**Figure 1: Simple regression representing concentrations of the fertilizer (PMR) versus snails` mortality**

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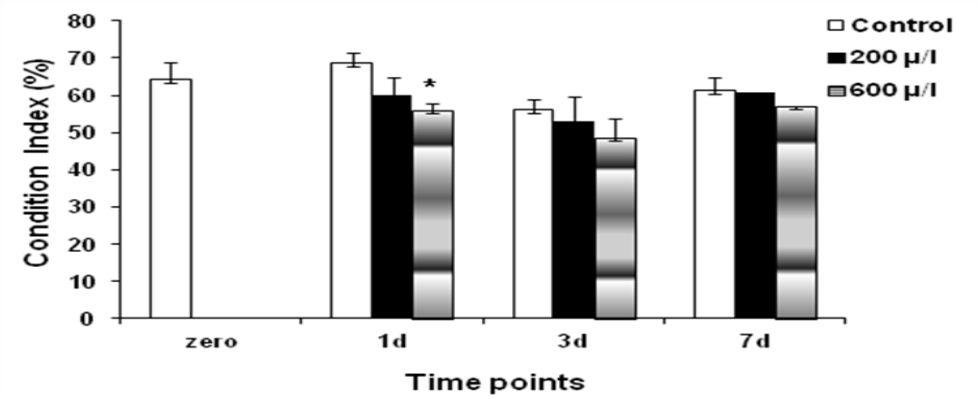


**Figure 2: Effect of the fertilizer (PMR) on adult *L. carinatus* condition index for 7 days of exposure. * indicates a significant difference from the control (ANOVA/Kruskal-Wallis, $P \leq 0.05$).**

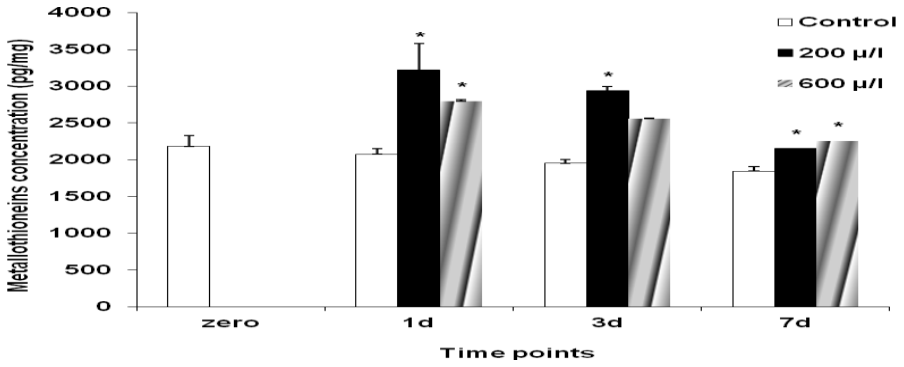


**Figure 3: Effect of the fertilizer (PMR) on adult *L. carinatus* metallothioneins for 7 days of exposure. * indicates a significant difference from the control (ANOVA/Kruskal-Wallis, $P \leq 0.05$).**

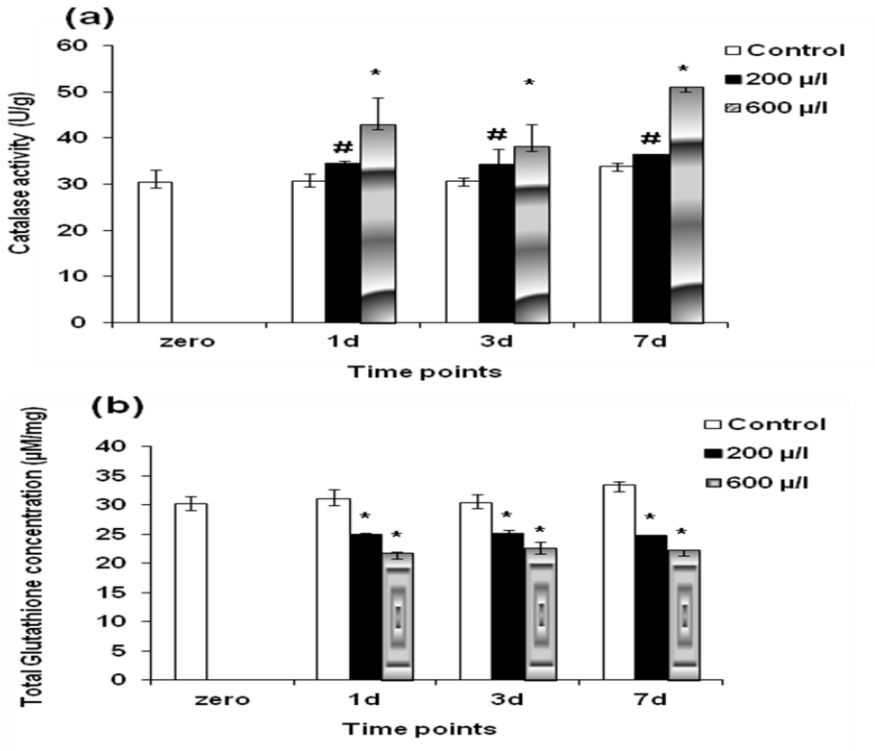


**Figure 4: Effect of the fertilizer (PMR) on adult *L. carinatus* antioxidants (a) CAT activity (U/g) and (b) GSH concentration (µM/mg) for 7 days of exposure. * indicates a significant difference from the control and # indicates a significant difference between treatments (ANOVA/Kruskal-Wallis, $P \leq 0.05$).**

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## DISCUSSION

The toxicity and oxidative stress caused by Caselio, the fertilizer was confirmed in the exposed snails. Snails from the control group were in better health with normal values of metal accumulation, metallothioniens concentration and antioxidants activity compared to the treated snails with Caselio, the fertilizer as expected. The results of this work recorded adverse effects and snails' mortality for longer exposure to the fertilizer.

The present results recorded molluscicidal effect of the fertilizer (PMR) on the freshwater snail, *L. carinatus*. However, the essential metals are necessary in small concentrations for normal biological processes in most organisms. Abnormally high/low concentrations of metals can cause death to organisms. Use of ammonium sulphate fertilizers decreased the pH of water, which results in accumulation of organic matter as a result of decreased microbial activity and reduced breakdown of plant and animal residues [15] Camargo et al. [44] recorded increased nitrates toxicity with increased concentration and time for the freshwater invertebrates, the crustaceans *Eulimnogammarus toletanus, Echinogammarus echinosetosus* and the insect larvae *Hydropsyche exocellata*. Abdel Mola and Abd El-Rashid, [45] recorded the lowest number of total zooplankton at Bahr El-Bakar station where sewage discharges are connected to Lake Manzala, Egypt.

The high concentration of the plant fertilizer (PMR) caused significant reduction in snails CI when compared to the control group (17.5 % reduction). For the rest of the time points, CI lost any significant difference but still less than the control group. Geffard et al. [46] found an inconsistency in the trend of CI between mussels from control and transplanted sites. They also recorded organ weight increase in control mussels compared with transplanted site mussels. On the other hand, in an earlier study, Veldhuizen-Tsoerkan et al. [47] found no effect of controlled stressors (aerial exposure and temperature) on the CI of the mussels from the polluted site.

The plant fertilizer, PMR caused increases in Zn and Mn concentration, but reduction in accumulation of Fe and disturbance in Ca content of the shell, operculum, foot and soft tissue. A significant difference was recorded between organs accumulation for the same metals by Tarique et al. [20], Kavun and Podgurskaya [19] found that mussels from contaminated sites have evolved active adaptation by increasing kidney function to regulate metal concentration in the organs to the permissible concentration. The increase of Ca content in investigated organs could be due to the disturbance of carbonic anhydrase which affected by pH of the surrounding medium [48]. Carbonic anhydrase is responsible for decalcification and negative control of Ca deposition and is rich in the epithelia of the mantle [49]. Lotfy [50] recorded Fe as one of the metals which was accumulated more in bivalve shells than gastropod ones. On the other hand, Zn was more accumulated in gastropod shells than bivalves`.

In the current work, soft tissues accumulated more metals than others organs except for Ca which was mainly in the shell followed by soft tissues then others organs. Sharshar et al. [51] recorded similar results in the freshwater crayfish, *Procambarus clarkia*. They recorded the highest accumulation of Cu in the gills followed by the muscles and then the carapace. However, other metals like Pb accumulated mainly in the carapace. The results of Yap and Cheng, [52] agreed with the results of the present work. They recorded increase in accumulation of Zn and decrease in Fe content in the soft tissues and the similar pattern of accumulation in the different tissues.

Zn, Fe and Mn accumulated mainly in the different tissues of the investigated snail, however, shell was the target of Ca accumulated followed by the other tissues. These results were in agreement with Yap and Cheng, [52] who discussed that the shell and operculum were classified were macroconcentrators for non-essential metals (Cd and Pb) and most of the soft tissues were macroconcentrators for essential metals like Cu and Zn. They explained that as soft tissues use the essential metals for the biological processes and shell, operculum accumulates unwanted chemicals or the shell accumulated other metal than Ca for substitution Ca ions [53]. In addition, the different distribution manner of metals in tissues was attributed to the binding sites of MTs [26]. Invertebrates can respond to toxicants exposure by mucous secretion to trap the toxicant molecules from entering the organism [54].

Metallothioniens (MTs) were significantly increased in the exposed snails to both concentrations, especially the low concentration at all time points of the experiment. The increase in heavy metal concentration in the cells stimulates the synthesis of apothioneins that can bind to the metal cations in a non-toxic form, thus reducing their deleterious effects on the cells [26]. Amiard et al. [28] reported that pre-exposed organisms to a metal resist oxidising stress better than unexposed ones, because MTs induction seems to limit the effects of hydroxyl (OH) and superoxide ($O_2^-$) radicals by scavenging them. Ungherese et al. [29] recorded a positive correlation between MTs induction and trace metals in the sandhopper, *Talitrus saltator* (Crustacea, Amphipoda) collected in the wild. Rainbow et al. [30] discussed the fate of metals inside the organism as a detoxified form by binding to the heat resistant proteins, metallothioneins or the non-detoxified form by the heat sensitive proteins, enzymes.

Catalase activity was significantly increased in the exposed snails at all time points. On contrary, total glutathione concentration was significantly decreased in the exposed snails. ZnONPs (32 g/ml) caused a significant reduction in glutathione concentration and increase in catalase activity of *Lymnaea luteola* [55]. CAT and SOD were activated but not GPx after exposure of Zebra Mussel, *Dreissena polymorpha* to Triclosan [35]. Radwan et al. [36] recorded a positive relationship between enzymatic antioxidants (CAT) and metals concentration but negative relationship with no-enzymatic ones (GSH) and metals. Decrease of glutathione concentration could be attributed to its consumption as a substrate for GST and GPx, which means a strong oxidative stress, is going on in the organism/or decrease of overall metabolism of the organism [35].

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## CONCLUTION

Shell, foot and soft tissue could be used as target organs for studied metals (Zn, Mn, Fe and Ca) accumulation of *L. carinatus*. However, operculum was not a target organ for that metals accumulation with consistent trend, which means organ specificity and selectivity to metals binding. Caselio, the plant fertilizer (PMR) caused oxidative stress in the digestive-gonad complex of *L. carinatus* that could be used as a biomonitor for pollution with agricultural products.

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