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Impact of Pollution by Industrial Metallic Dust on Bio-Accumulator Organism *Helix aspersa*

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Abstract: In this work, the impact of dust released by the metal complex of steel EL-Hadjar (Eastern Algeria) on bio-organism and bio-accumulator indicator of pollution *Helix aspersa* was assessed. The biomarkers of toxicity glutathione reduced (GSH), glutathione-S-transferase (GST) and catalase were followed up by colorimetric and spectrophotometric methods. The main results showed that the presence of metallic dust causes a significant reduction of GSH and activation of the detoxifying enzyme system which results in a significant increase in GST and catalase activity. Results show that the factory rejects pollute the environment; their effects are not only on the little animals but also on human and its whole metabolism and enzymes activity. It could be concluded that the species *Helix aspersa* is an excellent bio-indicator of environmental degradation, it is sensitive to the presence of heavy metals and this sensitivity was shown by metabolic changes (disruption of the synthesis of enzymes).

Key words: Pollution, GSH, GST, Catalase, Bio-accumulation, Metal dust, *Helix aspersa*

INTRODUCTION

Many human activities produce polluting substances, including heavy metals, which often have negative influences on the health of living creatures, especially humans [1]. More recently attention has been paid to the use of invertebrates for the assessment of ecosystem quality in aquatic environments [2] and terrestrial environments, the fauna, including snails (mollusks, gastropods, pulmonis) [3]. These mollusks are recognized as relevant environmental indicators [4,5] by their ability to accumulate trace elements most frequent namely Cd, Pb and Zn. This property has been exploited to use the snails as bio-indicators of metal pollution [6,7]. Among the species used snail *Helix aspersa*, is a model of choice, mainly due to its worldwide distribution, reflecting the adaptation to habitats, soil and varied climate and ease of farming [8]. The digestive gland (or hepatopancreas) always contains the highest concentrations of Cd, Pb and Zn [9], the digestive tract appears to play a role in storage of Cd, the high capacity of accumulation of trace elements metal among snails are related to the effectiveness of detoxification systems involving structures sequestration and intracellular compartments, but also their limited ability to excrete certain metals conditioned by the need to avoid excessive loss of water [10].

The objective of this study was to evaluate the effects of dust from industrial metals on bio-markers of environmental stress (GSH, GST and CAT) under laboratory conditions using snail *Helix aspersa* as a model.

MATERIAL AND METHODS

Biological Material: The biological material used was a terrestrial snail, the snail *Helix aspersa* collected from Guelma region (Eastern Algeria), (Zone considered unpolluted) Snails (average weight of 8.5 " 0.15 g) were cultivated in the optimal environmental conditions following photoperiods of 18h light/24h, temperature 20 " 2 EC, humidity of 80 to 95% and fed on food wheat flour. Snails were kept in transparent polystyrene boxes (23.5 x 16.5 x 10.5 cm) with perforated lid, each box containing a wet sponge to retain moisture. Power is supplied in Petri dishes regularly every 3 days [11,12].

The Metallic Releases: Metal dusts used in this study were collected directly from the paths of the steel complex. Chemical analysis by atomic absorption (Model JANWAY) was used to determine the composition of the dust. This analysis determined the presence of 7 heavy metals (Table 1).

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Table 1: Composition (ppm) of dust discharged by the electric steel plant 1 (ACE 1) and the electric steel plant 2 (ACE 2) of the steel complex of El-Hadjar [13]

Sample	Cu	Zn	Pb	Cr	Ni	Mn	Fe
Dust ACE 1	3.7	240	24	10	1.2	320	3000
Dust ACE 2	7	480	62.4	12	1.3	540	36000
Total	10.7	720	88.4	22	2.5	860	6600

Method of Treatment: The treatment of animals was performed by adding increasing concentrations of dust in the diet (wheat flour). Four concentrations (100, 500, 1000, 1500 g/g of diet) and control were selected. Snails were divided into 10 lots (5 snails/lot). The treatment lasted 4 weeks for 10 lots [12,14].

Dissection and Removal of the Hepatopancreas: After 4 weeks the snails were weighed, placed fasted for 48h to empty their gut and then dissected. After dissection, the hepatopancreas was removed, weighed and divided into 3 fragments:

- A sample for the determination of glutathione reduced (GSH).
- A sample for the assay of glutathione S-transferase (GST).
- A sample for the assay of catalase (CAT).

Measured Parameters: The catalase activity (CAT) was directly assayed by 240 nm method [15]. Glutathione reduced was estimated by the method of Weckberker and Cory[16]. The activity of glutathione-S-transferase (GST) was assayed at 340 nm by the method of Habig *et al.* [17].

Statistical Analysis: The results are represented as averages " standard error, using Student 't' Test, with the MINITAB software version 14.0 [18].

RESULTS

Effect of Metal Discharges on the Rate of GSH: The changes in the rate of GSH due to the presence of metal dust are illustrated in Figure 1. The presence of xenobiotics, the rate of GSH decreased significantly in the highest concentrations (1500 g/g) compared to controls (P=0.029).

Effect of Metal on the Release Rate of the GST: Figure 2 shows that the rate of GST increases in dose-dependent manner in treated snails. Statistical analysis revealed a significant difference between the rate of GST in controls and treated groups with the concentration 1500 g/g (P = 0.034).

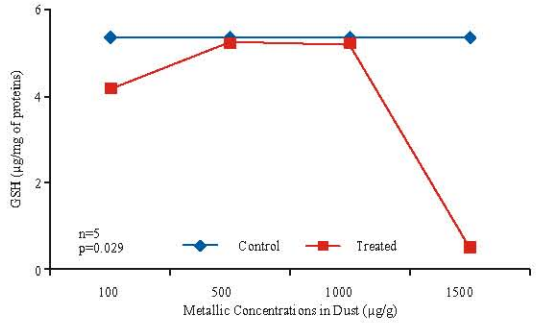

Fig. 1: Evolution of GSH based on metal concentrations in dust

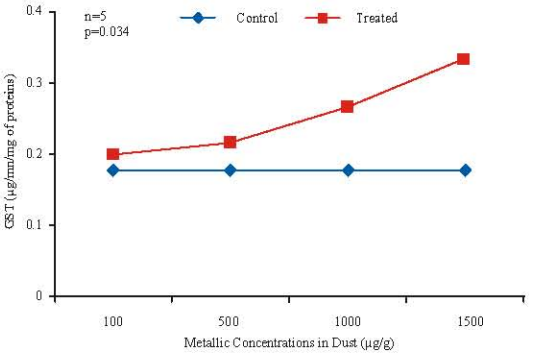

Fig. 2: Evolution of GST activity according to the metal dust concentrations

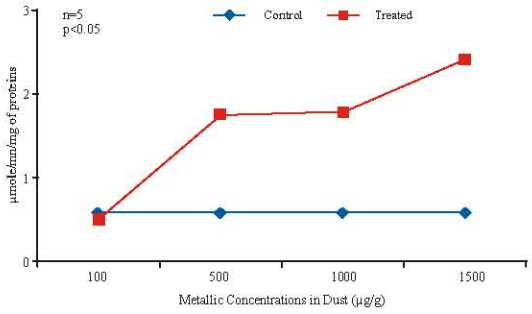

Fig. 3: Evolution of the Catalase in terms of concentrations of metal dust

Effect of Metal Discharges on Catalase Activity: Figure 3 represents the variations of catalase activity as a result of metal dust; results show that the presence of xenobiotics increases the activity of catalase in dose-dependent manner. Statistical analysis revealed a significant difference between control and treated groups with the concentration 500 g/g (P = 0000) and a significant difference in the concentration 1000 g/g (P = 0.05) and a highly significant difference in the concentration 1500 g/g (P = 0.01).

DISCUSSION

In thise present work, after treatment with metallic dust concentrations a significant decrease of GSH in the presence of high concentrations of metal dust (1500 g / g) was evident in the used snail model. This depletion can be explained by the direct link between metal and glutathione because glutathione has a carboxylic acid group, an amine group, sulfhydryl group (-SH) and two bypass peptide which may be involved in reactions with heavy metals [19]. Its functional group (-SH) then plays an important role in the binding to the metal. Indeed, many metals are characterized by a strong affinity towards thiol groups and form complexes with these chemical entities. This glutathione-metal interaction leads to the formation of thiyl radicals ($-S^0$). Although, these radicals are relatively stable and can interact with each other to form disulphide bridges not radical, they can also react with oxygen and generate reactive oxygen species [20] and causes the appearance free radicals responsible for significant tissue damage [21]. This highlights the important role of glutathione in the management of a metallic stress. On the other hand, the reactions of metals with glutathione are reflected by the formation of complexes from the oxidation of GSH [15]. According to the study of Christie and Costa [22], metals that cause oxidation of GSH are Cu, Cd, Mn, Fe and Cr. While stable complexes with GSH are formed by the Zn, Cd, Hg, Pb and Ni and these two reactions could explain the decrease of glutathione. On the other hand, the GSH is responsible for the synthesis of metallothioneins. These are specialized in metal chelating, which explains the decrease of GSH. Also,the reduction of GSH may be attributed to its usage by the GST in the conjugation reaction,. These results were consistent with work of Chandran *et al.* [23] and, Regoli *et al.*[24]. The present results show a significant increase in the GST in the hepatopancreas in the presence of metallic dust which may be a response to oxidative stress caused by the presence of heavy metals in the body, whereas, biotransformation enzymes are among the first reactors due to the presence of xenobiotics in a living organism [25]. This increase indicates a high rate of conjugation of metal particles with glutathione. Induction of GST has been reported in mussels exposed to heavy metals [26] as well as in bivalves *(Mytel guyanensis)* collected from sites contaminated by heavy metals [27].

The current results have highlighted an increase in catalase activity in the treated groups, probably due to the intensification of anti-oxidant activity in the cells of hepatopancreas; according to Halliwell and Gutteridge [28] the increase of oxidative stress increases the activity of antioxidant enzymes in animals. Indeed, the catalase activity is a transformation of hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). However, the production of hydrogen peroxide is induced by the presence of exogenous compounds to the body as is the case for metal [29,30], this derivative reagent oxygen can cause oxidation of macromolecules (DNA, lipids and proteins) [31], is catalase with superoxide dismutase (SOD) the first line of defense against oxidative stress [32]. Unlike other bio-markers (GSH and GST), it was found during this study that the significant effects of the catalase activity was observed in the lowest concentrations that may be due to the sensitivity of this bio-marker. In fact, catalase is considered as one of the biomarkers most significantly oxidative stress marker and particularly toward chemical pollutants in the aquatic environment [33]. In *Helix aspersa*, the catalase activity showed a core of about 5 to 10 times higher than in marine mollusks [34,15]these high values of catalase activity suggest the stimulation of the system of antioxidant defense in the hepatopancreas.

It could be concluded that the species *Helix aspersa* is an excellent bio-indicator of environmental degradation, it is sensitive to the presence of heavy metals and this sensitivity was shown by metabolic changes (disruption of the synthesis of enzymes). The evolution of bio-markers of stress (detoxification enzyme) confirmed the toxicity of metallic dust, which is manifested by the significant decrease of glutathione, as well as triggering a series of enzymatic processes such as the GST and the activity catalase-known for their role in detoxification.

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