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Toxicity of metal dust from Annaba steel complex (Eastern Algeria) on the morpho physiological parameters of the snail Helix aspersa

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ABSTRACT

In this study we were interested in assessing the impact of metal dust collected at the steel complex of El-Hajar-Annaba (Eastern Algeria) and their effects on organisms and organic bioaccumulators indicators of pollution *Helix aspersa*, by contamination of food with different concentrations of metal dust. The parameters studied were: morpho physiological parameters (weight change snails, organ weights (hepatopancreas and kidney) weight of soft tissues, weight and diameter of the shell). The main results show that the presence of metal dust caused a loss of weight in snails, a dose-dependent decrease of organ weights especially the hepatopancreas and kidney and decreased the weight of soft tissues and decreased diameter of the shell and weight.

Key words: Pollution, Bio battery, metal dust, weight, shell, soft tissues, hepatopancreas, kidney, *Helix aspersa*

Introduction

Environmental pollution by metals has become the most important problems in the world. Environmental poisoning by heavy metals has increased in recent decades due to extensive use of heavy metals in the process of agriculture, chemical and industrial, becoming a threat to organic matter. These heavy metals often have negative influences on the health of living beings and especially of man [3]. The evaluation of risk factors associated with these pollutants is a major problem, which defines standards and threshold concentrations of pollutants in the environment through chemical analysis of water, soil and air. It is therefore necessary to have indicators of environmental stress, which are sets of an organism or organisms that are used as sentinels in studying the physiological changes, biochemical, environmental, that affect them. These creatures are very sensitive to contaminants present interest to lend more readily than human studies of the effects of pollutants and help to identify chronic or sudden pollution [15,21].

For many years, gastropod molluscs are known for their great capacity for accumulation of trace metals the most common, namely Cd, Cu, Pb and Zn. This property has been exploited to use the snails as model organisms to study the kinetics of accumulation and detoxification [33,30]. Among the species of snail *Helix aspersa* is used, the preference of this species is mainly due to its worldwide distribution, reflecting the ability to adapt to habitats, soil and varied climates and its ease of breeding [19]. Digestive gland (or hepatopancreas) has consistently higher concentrations of Cd, Pb and Zn [10,9,13], the gut appears to play a role in Cd storage, high capacity for accumulation of the metal elements traces (MET), in snails are related to the effectiveness of detoxification system structures involving kidnapping and intracellular compartmentalization but also to their limited capacity to excrete some metals including packaged the need to avoid excessive loss of water [14].

The objective of this study was to evaluate in laboratory conditions the effects of metal dust from industrial sources on the morpho-physiological parameters in an organism bioaccumulation of heavy metals the snail *Helix aspersa.*

1 - Materials and Methods:

1-1 Biological Material:

the biological material used is a terrestrial snail: the snail *Helix aspersa* collected in the region of Guelma (North - East Algeria).Snails (average

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weight of 8.5 ± 0.15 g) are high in the optimal environmental conditions following photoperiod 18h light / 24h, temperature 20 ± 2 ° C, humidity 80 to 95% food in flour wheat, they are distributed in transparent polystyrene boxes (23.5 x 16.5 x 10.5 cm) with perforated lid, each box contains a wet sponge to maintain moisture. Power is supplied in Petri dishes regularly every 3 days [17,7].

1-2 -Chemical Material:

1-2-1-The Metal releases:

Metal dust used in this study were collected at steel complex EL-Hajar, The latter is located 13Km from the city of Annaba on the road No. 44 (North - East Algeria), a chemical analysis by atomic absorption was used to determine the composition of this dust. This analysis identified the presence of 07 heavy metals listed in the table (02).

Table 2: Composition in ppm Dust rejected by the EAF 1 (ACE 1) and EAF 2 (ACE 2) of the steel complex of El Hajar-Annaba). (Kleche, 2002).

sample	Cu	Zn	Pb	Cr	Ni	Mn	Fe
Dust ACE1	3,7	240	24	10	1,2	320	3000
Dust ACE 2	7	480	62,4	12	1,3	540	3600
Totale	10,7	720	88,4	22	2,5	860	6600

1-3 Method of Treatment:

Treatment of animals was performed by adding increasing concentrations of metal dust in the diet (wheat flour). We selected four concentrations and a control medium (100, 500, 1000, 1500 µg / g of food)) . Snails are divided into 10 lots (5 snails / lot.). The treatment lasted 4 weeks for 10 lots [75].

1-4 Preparation and animal sacrifice:

After each treatment period, snails were sacrificed by freezing at -80 ° C to the end of testing, without prior fasting that could change the expression levels and then dissected, the shells are removed and the kidney and Hepatopancreas were excised, washed with physiological saline(0.9%).

1-5- Search physiological biomarkers:

Three criteria can be used:

The fresh weight of the snail (shell + soft tissue) is measured using a precision balance every week (for 4weeks). This test is recommended to evaluate the effects of a weekly metal dust and cadmium on adult snails *Helix aspersa*. The weights are always performed before cleaning of the test chamber [17], The diameter of the shell is measured with a caliper every week (for 4weeks). The measurement must be done carefully to avoid damaging the edges of the shells of snails, very fragile in snails. As the fresh weight, measurements of the diameter of shell used to evaluate the growth of animals at any time. This parameter is used to monitor the development of individual weights (Chevallier, 1992) and the fresh weight of the shell, the soft tissues and fresh weight of both organs (Hepatopancreas (H) and kidney (K) may be made only after the sacrifice of animals at the end of each treatment period using a precision balance [7].

1-6-Stastical study of results:

The statistical analysis is performed by the Kruskal-Wallis test for comparing two samples (control and treated). This test is performed using a data analysis software: Minitab (Version 14.0)[12].

2-Results:

2-1 - Effect of metal dust on the evolution of the weight of snails:

Figure (01) shows the evolution of the mean weight of snails over time. We note that in controls and treated with the concentration 100µg / g, the weight tends to increase with time. However, in treated concentrations (500.1000 and 1500µg), a dose-dependent decrease of the weight is emphasized from the first week. Statistical analysis shows a significant difference between the weight of witnesses and treated with the concentration (1500µg) to the 2nd and 3rd week and a highly significant difference in the 4th week. The difference between the weight of the treated and control is significant at the concentration 1000µg / g in the 4th week.

2-2-Effect of metal dust on the evolution of the diameter of the shell snails:

Figure (02) shows the evolution of the diameter of the shell of snails over time. We note that in controls and treated with the concentration (100µg), the diameter of the shell tends to increase with time. However, in treated concentrations (500.1000 and 1500µg), we find a dose-dependent decrease in the diameter of the shell from the first week. Statistical analysis shows a significant difference between the diameters of the shells of witnesses and treated with the concentration 1500µg / g in the 1st week and treated with the concentration (500µg) at the 2nd weeks. We also note a very highly significant difference for those treated with the concentrations (500.1000 and 1500µg) at week 4.

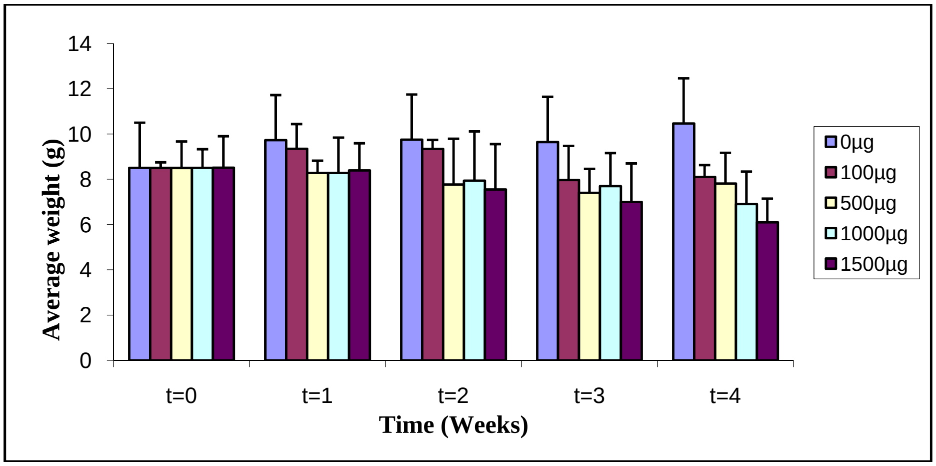

Fig. 1: Effect of metal releases of the evolving weight of *Helix aspersa* with time.

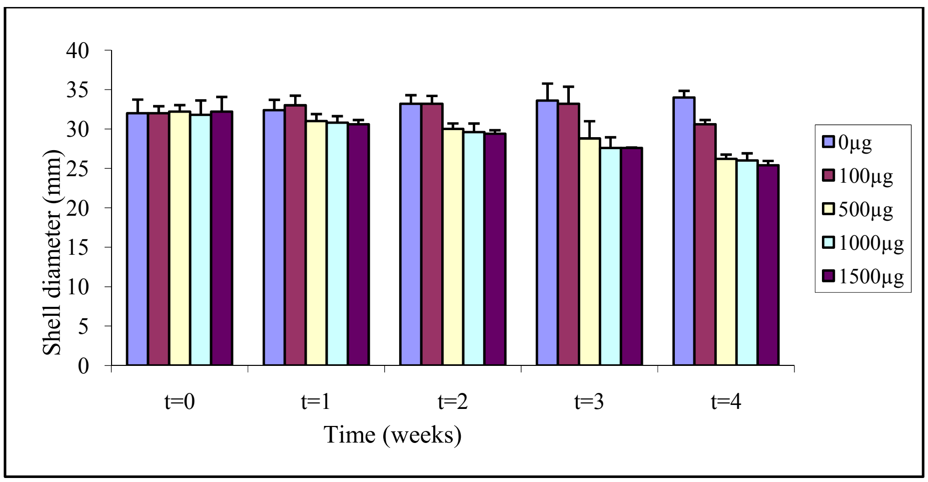

Fig. 2: Effect of metal releases on the evolution of the diameter of the shell of *Helix aspersa* with time.

2-3-Effect of metal dust on the weight of soft tissues:

Figure (03) shows variations in the weight of the fabric soft in the presence of metal dust. We find a dose-dependent decrease in the weight of soft tissues from the first concentration in the presence of xenobiotic. On the other hand, statistical analysis revealed a significant difference between control and treated is observed especially at the concentration (100µg /g) and a very highly significant difference between witnesses and treated concentrations (500.1000, 1500µg / g).

2-4-Effect of metal releases on the weight of the shell:

Figure (04) shows variations in the weight of the shell in the presence of metal dust. Note that the weight of the shell decreases slightly in snails treated with 100µg concentration compared to controls, but the weight of the shell decreases in a dose - dependent is significant in snails treated with the concentration (1000µg) by compared to controls. In the treated concentration (1500µg) the difference is highly significant.

2-5-Effect of metal releases of organ weights (hepatopancreas and kidney):

Figure (05) shows variations in the weight of the hepatopancreas and kidney in the presence of metal dust. We note that in the presence of xenobiotic weight of two bodies decreases and not significantly with ($P \geq 0.05$) in snails treated with concentrations (100.500, 1000 and 1500 µg / g) compared to controls.

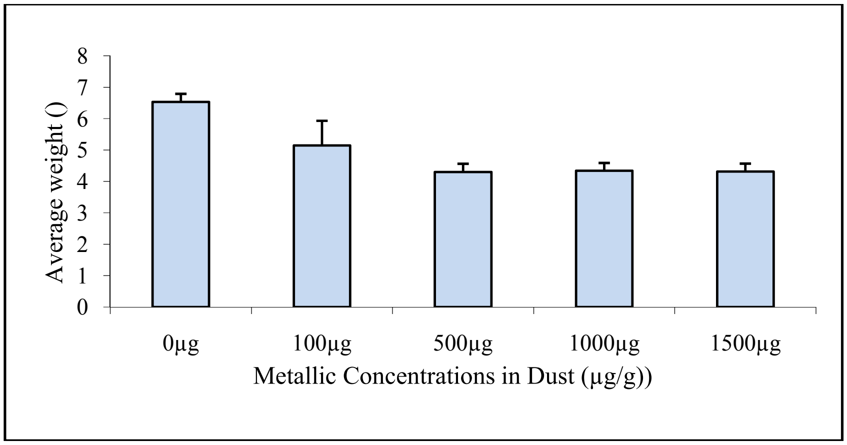

Fig. 3: Changes in the average weight of the soft tissues of snails *(Helix aspersa)* as a function of increasing concentrations of metal

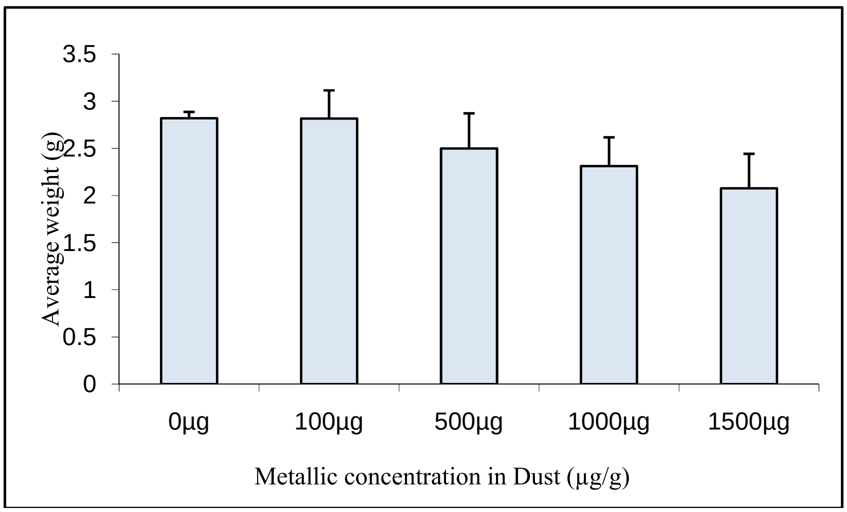

Fig. 4: Evolution of the average weight of the shell snails *(Helix aspersa)* as a function of increasing concentrations of metal dust.

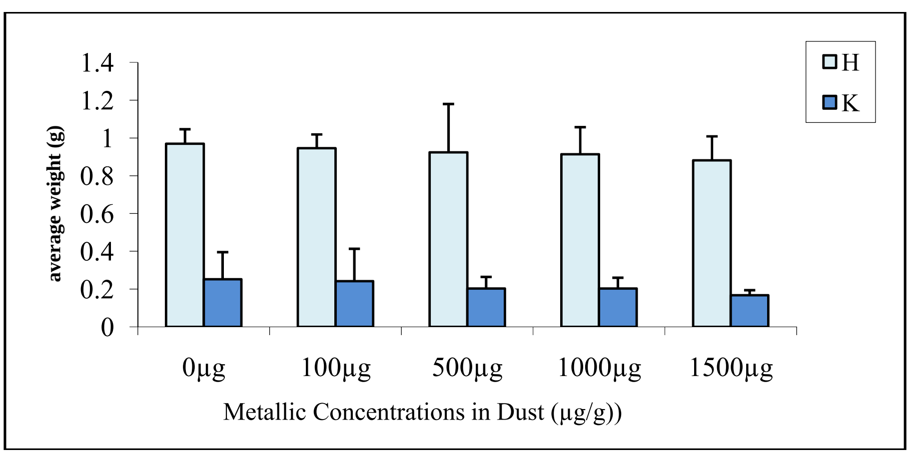

Fig. 5: Evolution of the average weight of organs (hepatopancreas and kidney) of snails *(Helix aspersa)* as a function of increasing concentrations of metal dust.

Discussion:

In the case of metal pollutants, the digestive tract contributes to a very large, probably greater than 90% in the wild, the absorption of Cd; this is consistent with the observations of Hopkins [22] on the preponderance of intoxication in the digestive tract of terrestrial invertebrates by metals.

In our work we have chosen as a model biological *Helix aspersa* and we first noticed a decrease in body weight in animals treated with different concentrations of metal dust which is a first indication of toxicity. This result is consistent with the work of Laskowski and Hopkin [26] on the effect of Zn, Cu and Cd on *Helix aspersa* and Coeurdassier and *al*., [5] who studied the effect of chromium on *Helix aspersa* . This weight loss can be explained by the decrease in food consumption that we have seen particularly in animals treated with high concentrations of metal dust and Cadmium (1000 and 1500µg). As stated Laskowski and Hopkin [26]. On the other hand, Van Straalen and *et al.* [32] suggest that these animals are able to regulate the amount of metals in their body mass with a consequent decrease in food consumption at high doses of xenobiotics.

Regarding the weight of the shell, we note a decrease dose - dependent in the presence of metal dust. The (MET) are known for their direct toxicity in conjunction with, for example, irreversible damage to certain enzymes or DNA, they can also indirectly disrupt important physiological processes by competing with essential elements such as calcium (Ca) [1,28]. In these individuals, the depletion of Ca, in conjunction with the inhibition of shell growth, may be responsible for the shift of four weeks in the laying cycle, time probably necessary for the adjustment of dynamic Ca [7].

Concerning the evolution of the diameter of the shell, we note a decrease dose - dependent on this factor in the presence of metallic dust, these results are consistent with those of Coeurdassier and *et al.* [7], which showed an inhibition of the diameter of the shell of snails after exposure to dimethoate (organophosphate-based pesticide). Calcium plays an important role in the development of the whole body of snails, it has been shown in the *Helix aspersa* [18] and In Achatina fulica [23] explains this effect by increasing the thickness and mass of the shell, lead is known by its interference with Calcium shellfish and can interfere with enzymatic reactions requiring calcium as a cofactor [2]. In addition, interference and competition between Ca and metals (Cd ...) were also observed in several studies [11,28]. Our results are consistent with the work of Gimbert and *et al.* [16] which showed a decrease of 30% of the mass of the shell of snails exposed to artificial soil contaminated with cadmium. These results are also consistent with the work of Beeby and *et al.* [1].

The other important factor considered in this section for monitoring the weight of soft tissues, we demonstrated a dose-dependent decrease in the weight of soft tissues in the presence of metallic dust, this can be explained by competition between calcium ions and metal ions studied as suggested Coeurdassier and *et al.* [7], which showed an inhibition of the weight of the soft tissues of snails after exposure to dimethoate (organophosphate-based pesticide).

To better support our result, we have chosen to follow the evolution of two main bodies, namely the hepatopancreas and kidney. Our results show a perturbation of the weight of these organs. These results are consistent with those of Marigomez and *et al.* [27] showed a 50% reduction in weight of the digestive gland of the ingots in the presence of xenobiotics.

On the other hand, de Gomot Vaufleury and Kerhoas, [20] has found throughout the body, concentrations of 200 micrograms Cd caused a significant inhibition of development of the genital tract *Helix aspersa*. Even in mammals, the presence of metals is the cause of a decrease in Soft organ weights (liver and kidney) including rabbits [31].

Conclusion:

At the end of this chapter, we can conclude that the species *Helix aspersa* is sensitive to the presence of heavy metals; the sensitivity was manifested by a disturbance in the development of snails exposed through a decrease in body weight, weight organs and a decrease in the diameter of the shell and weight. Ultimately, our experiments show that snails respond well to the criteria of bioindicators recalled by Hopkin (1993) to participate in the biomonitoring (biomonitoring).

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