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Study of the behavior/adaptation of non-target biological models exposed to multiple pollution

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Key words: *Helix aspersa,* Polycyclic aromatic hydrocarbon, Biomarker, Bio indicators, Bioaccumulator.

Abstract

In this study, we were interested in assessing the impact of leachate leaves three plant species (*Acer monspessulanum, Populus nigra, Salix babylonica)* taken near petrochemical complex (Skikda N-E. Algeria) on *Helix aspersa*. The first results show that treatment with leachates plant leaf exposed to polycyclic aromatic hydrocarbon emissions by petrochemical complex cause a dose-dependent inhibition of the growth of the shell *Helix aspersa* (weight and diameter). On the metabolic level, leachates cause a significant increase in protein at hepatopancreas and kidney and significant increase in Catalase activity and Glutathione, glutathione S-transferase.

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Introduction

The emission of pollutants into the environment by human activity been a recent awareness primarily through the permanent action of advocacy by the international scientific community and public opinion increasingly informed, concerned and involved.

Due to the rapid development of industrial activities (especially oil refining), the increasing number of vehicles, increased use of energy (combustion of coal, oil, wood, and gas), large quantities of aromatic hydrocarbons (PAH) are emitted into the environment.

These sources emitting pollution are often located close to residential areas of man, which creates a major public health problem that requires the use of biological systems and alarm biomonitoring primarily focused on the early detection of signs reflecting alterations rather than conducting an inventory too late (Viard, 2004).

To do this, organizations bio-accumulators/bio-indicators of pollution such as amphibians and gastropods are used to provide information on the environmental characteristics of their habitat, the bioaccumulation of micropollutants in their bodies or variation sensitive physiological processes to xenobiotics (lam *et al.*, 2003).

The accumulation is a phenomenon by which a substance in the environment, enters a body even if it has no metabolic role, even if it is toxic to the latter (Ramade, 1992), it reflects a state of dynamic equilibrium between the processes of absorption, storage and excretion of (Rainbow *et al.,* 1993), and is used to indicate the level of contamination of a medium.

In plants, this phenomenon is known as the phytoaccumulation which is a mechanism to extract and accumulate pollutants in plants but that they do not undergo degradation or transformation. The phytoaccumulation concerns a majority of ETM and organic pollutants (Meagher, 2000; Susarla *et al.*, 2002). This mechanism is based on the ability of plants to extract and accumulate pollutants in soils. This results in absorption and accumulation of pollutants in plant tissue (Vila, 2006).

Materials and methods

• The aim of the study was to evaluate the capabilities of phytoaccumulation 3 plant species by using leachate their leaves as a source of PAH pollution in the context of biomonitoring, and to assess the toxicity of PAHs using a model bioindicator of pollution is the terrestrial gastropod *Helix aspersa* (*Cornu Cantareus aspersus or aspersum) (*Müller, 1974.). *aspersa subspecies,* commonly known as Petit-Gris.

The species selected in the context of our research *Acermonspessulanum, Populus nigra, and Salix babylonica* widely represented in the study area (Skikda).

A contaminated site is defined as a site of actual or potential permanent risk to human health or the environment due to pollution resulting from current or former (Viard, 2004) human activities.

We chose leaves 3 plants located near the petrochemical complex. These leaves were kept separately in freezer bags. We also collected water samples.

Preparation technique for batch leaching

In our experiments, we used two types of treatment: with fresh leaves and dried leaves, and they were divided into two series:

1. The first set is used to determine the relationship between fresh weight and dry weight of leaves after they were individually weighed a few hours after harvest (fresh weight: PF) and then dried in an oven at 40°C for 48 hours and immediately reweighed (dry weight: PS).

$PS = a\ PF + b$

2. The second series is divided into two lots:

• Abatch oven dried at 40°C for 48h batch is dry.

• A lot kept cool at 5°C in freezer bags until use is the fresh batch. (Ben slimane, 2004).

Leaching of leaves

The leaves of the two batches were weighed in batches of 10g, and then each batch was leached at 25°C separately in beakers containing 1 liter of tap water.

The total number of tested batches of 3 plant species (*Acer monspessulanum, Populus nigra, Salix babylonica*) × 2 treatments (fresh and dried) × 3 leaching time (12h, 24h, 48h) or 18 lots. (Ouaritini, 2013).

The treatment was performed gastropods by spraying water from the leaching of the sheets (18 units) and the water withdrawn from the site (a batch) and that two times per day.

We treated 19 lots and 1 lot kept as a control. Snails are divided into 20 lots of 3 snails/lot. The treatment lasted 2 weeks for 20 lots.

A total of 20 sheets of each species we have determined the relationship between the dry weight and fresh weight given by regression equation PS = a PF + b (Table 01)

a: coefficient of coordination.

b: constant.

Table 1. Relationship between dry weight and fresh weight of the leaves of three plant species studied**.**

plant species	Regression Equation	Correlation coefficient
Acer monspessulanum	PS= 3.36439PF – 5.96993	0.921
Populus nigra	PS= 0.880830PF – 1.52640	0.778
Salix babylonica	PS= 0.63933PF – 0.464062	0.908

Determination of catalase (CAT) activity

The CAT activity was determined spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 (Regoli et Principato, 1995). The result was expressed as µmol/ min/ mg of proteins.

Glutathione (GSH) content

GSH content was determined using the method of (Wechbeker and Cory, 1988). Cells are mixed in 1ml EDTA (0.02 M). The homogenate was centrifuged. The assay mixture contains 1ml tris/EDTA buffer (0.02 M, pH 9.6), 0.025 ml of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and the sample. The absorbance was measured at 412 nm and the amount of GSH was expressed as µmol/mg of proteins.

Determination of glutathione S-transferase (GST) activity

The activity of GST was measured according to the method of Habig *et al.,* 1974. The final reaction contain 1,2ml CDNB (1mm)/GSH (5mm) and the sample. The absorbance was measured spectrophotometrically at 340 nm. The result was expressed as µmol/ min/ mg of proteins.

Results

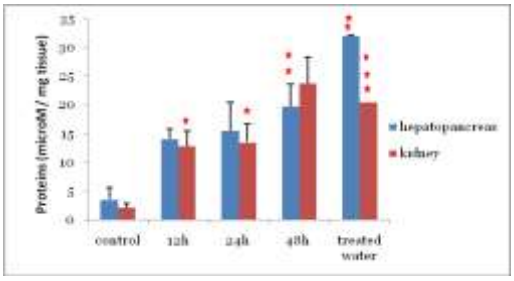

Fig. 1. Effect of treatment of leachates Acer fresh leaves on changes in catalase activity in hepatopancreas and kidney of *Helix aspersa.*

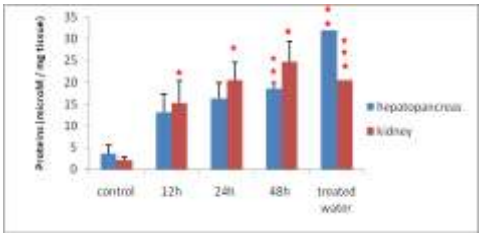

Fig. 2. Effect of treatment of leachates Acer dried leaves on changes in catalase activity in hepatopancreas and kidney of *Helix aspersa.*

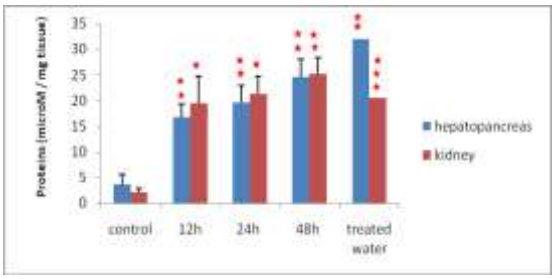

Fig. 3. Effect of treatment with leachates of fresh poplar leaves on changes in catalase activity in hepatopancreas and kidney of *Helix aspersa*.

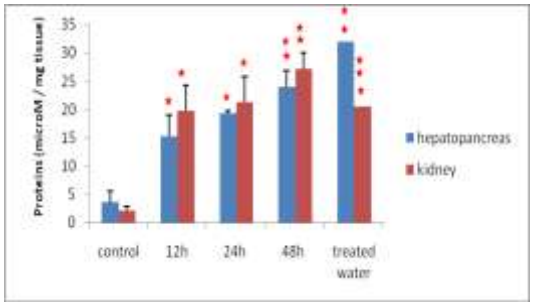

Fig. 4. Effect of treatment of leachates Poplar leaves dried on changes in catalase activity in hepatopancreas and kidney of *Helix aspersa*.

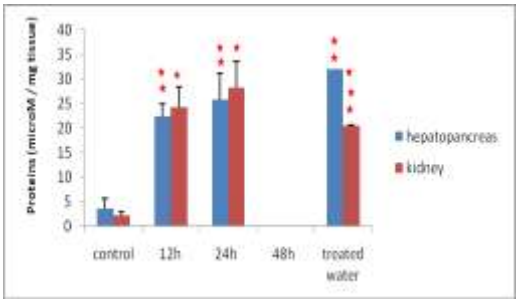

Fig. 5. Effect of treatment of leachates on fresh leaves of Salix variations of catalase activity in the kidney and hepatopancreas *Helix aspersa*.

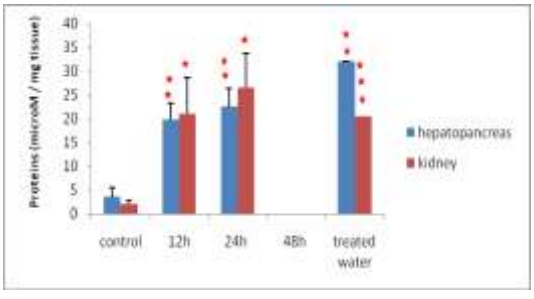

Fig. 6. Effect of treatment of leachates sheets Salix dried over variations in the level of catalase activity and kidney hepatopancreas *Helix aspersa*.

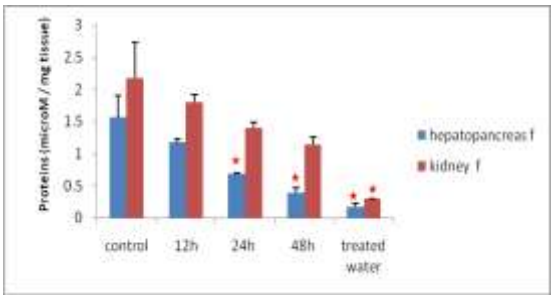

Fig. 7. Evolution of GSH following treatment with leachate from fresh leaves Acer.

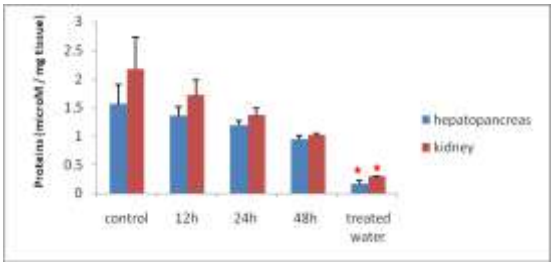

Fig. 8. Effect of treatment of leachates Acer dried leaves on changes in GSH level hepatopancreas and kidney of *Helix aspersa*.

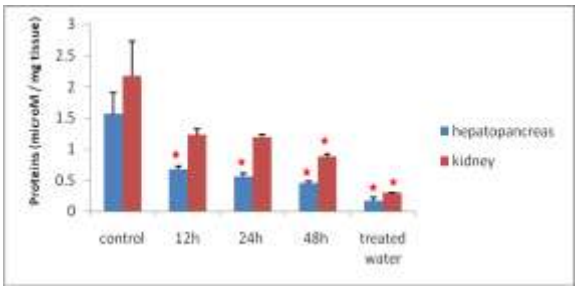

Fig. 9. Effect of treatment with leachates of fresh poplar leaves on changes in GSH level hepatopancreas and kidney of *Helix aspersa*.

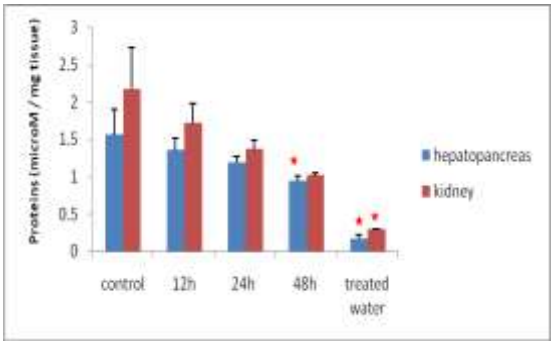

Fig. 10. Effect of treatment of leachates Poplar leaves dried on changes in GSH level hepatopancreas and kidney of *Helix aspersa*.

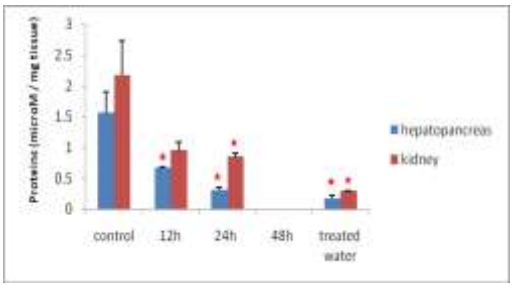

Fig. 11. Effect of treatment leachates salix fresh leaves on changes in the rate of GSH in hepatopancreas and kidney of *Helix aspersa*.

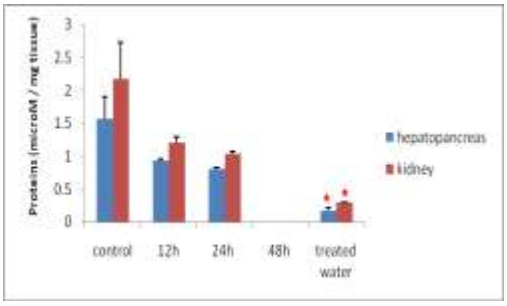

Fig. 12. Effect of treatment leachates Salix leaves dried on changes in GSH level hepatopancreas and kidney of *Helix aspersa*.

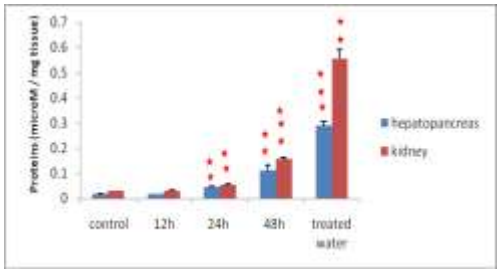

Fig. 13. Effect of treatment of leachates Acer fresh leaves on changes in total protein content in hepatopancreas and kidney of *Helix aspersa*.

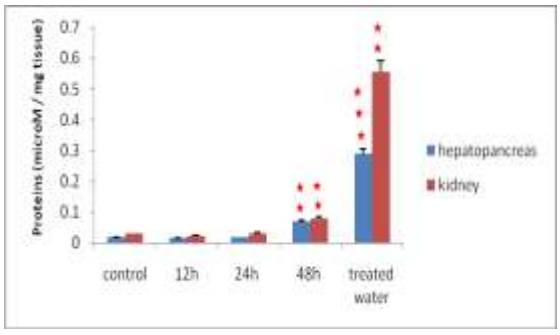

Fig. 14. Effect of treatment of leachates Acer dried leaves on changes in the rate of GST in hepatopancreas and kidney of *Helix aspersa*.

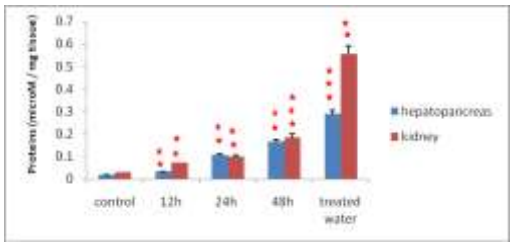

Fig. 15. Effect of treatment with leachates of fresh poplar leaves on changes in the rate of GST in hepatopancreas and kidney of *Helix aspersa*.

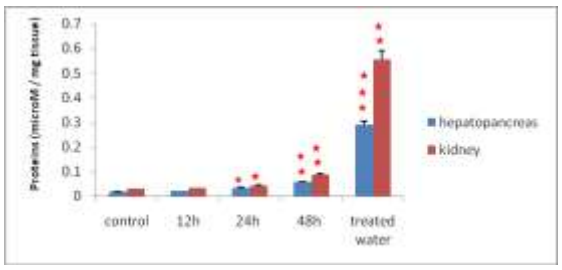

Fig. 16. Effect of treatment of leachates Poplar leaves dried on changes in the rate of GST in hepatopancreas and kidney of *Helix aspersa*.

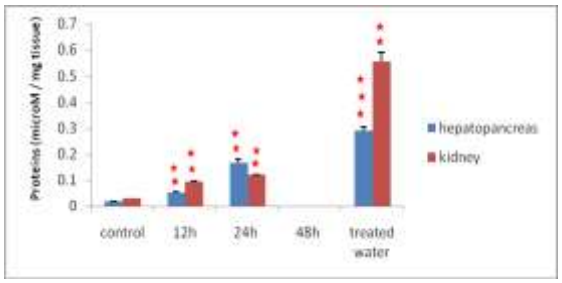

Fig. 17. Effect of treatment leachates salix fresh leaves on changes in the rate of GST in hepatopancreas and kidney of *Helix aspersa*.

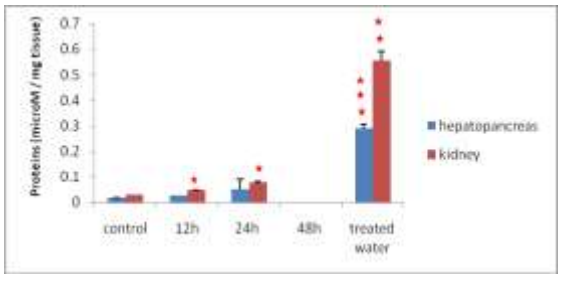

Fig. 18. Effect of treatment leachates Salix leaves dried on changes in the rate of GST in hepatopancreas and kidney of *Helix aspersa*.

* Snails treated leachate Salix babylonica 48 h have a mortality rate of 100%.

Discussion

The study of the toxicity of leachates dried and fresh leaves, we showed that the longer the duration of leaching increases and increases its toxicity, the results show a significant and dose-dependent growth inhibition in animals treated by different leachate (3 plants) and that according to the leaching time, which constitutes a first index of toxicity this is consistent with the work (Gomot *et al.*, 2000), which showed inhibition of growth in *Helix aspersa* exposed to pentachlorophenol, trichlorophenol and naphthalene or its spraying leachate.

Indeed, the results show a dose-dependent relationship of the weight vis-à-vis the leaching time.

We also demonstrated a dose - dependent decrease of changing the diameter of the shell in animals treated with different leachate (3 plants) and in the leaching time. These results corroborate those of (Coeurdassier *et al.*, 1957), which showed an inhibition of shell diameter of snails after exposure to dimethoate (organophosphate pesticide base). These results are also consistent with the work of (Laskowski *et al.*, 1996; Coeurdassier *et al.*, 2000) which states that in a polluted environment, exposed animal undergoes a growth inhibition. This inhibition of growth (weight, diameter) can be explained by the decrease in food consumption as required (Bibic *et al.*, 1997), and confirmed by (Notten, 2006.) or with (Atailia 2009, Grara, 2011) on the *Helix aspersa* species.

In our work, we have demonstrated that the rate of total protein in both organs (hepatopancreas, kidney) increases in a dose-dependent manner following treatment with different leachate (3 plants). These results are in the same direction as those of (Peccini *et al.,* 1994; Masaya *et al.*, 2002) which showed a significant increase in total protein content under the effect of a chemical stress in different biological models (tadpoles, ciliated protists, rabbits).

The antioxidant defense system is present in all aerobic cells and neutralizes the intermediate chemical reactions produced by endogenous and/or track the metabolism of xenobiotics. The antioxidant activity of the system or may undergo an increase in the inhibition effect of a chemical stress (Winston *et al.*, 1991.).

In our work, we measured the catalase activity that can be modified and or disturbed during toxicity. Our results showed a significant and dose-dependent increase of catalase activity in individuals treated with different water leaching, probably due to increased antioxidant activity in the cells of the hepatopancreas and kidney. According to (Halliwell *et al.,* 1995.), increased oxidative stress enhances the activity of antioxidant enzymes in animals. The catalase has a role in protecting the body against damages of oxidative stress (Wood *et al.,* 2001; Suntres, 2002).

We found in our study that significant effects of catalase activity were observed dice lower concentrations and this may be due to the sensitivity of this biomarker. Indeed, catalase is considered one of the most sensitive biomarkers of oxidative stress (Hopkin, 1993).

This study focused on the impact of three plant species on a biological model terrestrial gastropod *Helix aspersa*, this study shows the value of *Helix aspersa* as a model species of terrestrial pulmonate gastropod for assessing the toxicity of PAHs in laboratory tests and monitoring of terrestrial receptors using these organisms in a process biosurvaillance. It is clear that the species Helix aspersa is an excellent bio-indicator of environmental degradation, it is particularly sensitive to pollution by inorganic or organic pollutants (HAP).

At the end of our work, we can say that: the Helix aspersa is an excellent bioindicator of environmental degradation and meets the criteria Bioindication recalled by (Hopkin, 1993). It is sensitive to the presence of PAHs This sensitivity is manifested by an

inhibition in the development of the physiological parameters, accompanied by increased production of free radicals, which results in a disturbance of the biochemical metabolism (increased protein synthesis total), and by the triggering of a battery of enzymatic processes, such as the catalase activity known for its role in detoxification.

The plants used have capacities phytoaccumulation seen the effects of their leachates Helix aspersa, so we can infer that the plants have storage capacity for PAH. We can say that each species of plants used for different degrees of accumulation. Salix babylonica has the largest capacity phytoaccumulation monitoring Populus nigra and finally Acer monspessulanum whose capabilities accumulation are the lowest.

According to (Haddy, 1993.) the longer leaching increases, the amount of polyphenolic substances increase.

The leachates of fresh leaves are more toxic than those dried leaves, it would probably be due to the fact that PAH are more easily leached when they are dissolved in the liquid fractions of leaves (Petersen *et al.*, 1974).

Snails treated water taken from the site studies are most affected by toxicity. This toxicity is due to the fact that water taken from the soil would be saturated with PAH, it may have the explanation that the ground is covered by leaf litter that falls from year to year so the concentration in the upper soil layer is very high PAH.

The free radicals produced are responsible for tissue damage and can lead to cell death (Grara, 2011) as in our case gastropods treated leachate Salix babylonica after 48 hours of leaching.

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