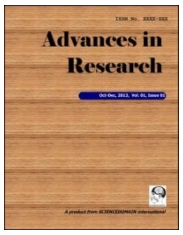

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Evaluation of the Effect of Oxidative Stress Generated by Cadmium (Cd) in the Yeast *Saccharomyces cerevisiae*

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Authors' contributions

This work was carried out in collaboration between all authors. Author FK designed the study, wrote the protocol and wrote the first draft of the manuscript, managed the literature searches, analyses of the study performed the spectroscopy analysis. Author NG managed the experimental process. Authors AB, IT and KB realized the practical and statistical study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Inhibition of the mitochondrial electron transfer chain and induction of reactive oxygen species (ROS) production are one of the roots of cadmium (Cd) toxicity. Our work concerns the study of the effects of a heavy metal; Cadmium (Cd) on the biochemical parameters, of microorganism bioaccumulator and bioindicators of pollution *Saccharomyces cerevisiae*. The main results show that the presence of Cadmium affects the growth of yeast, and biochemical assays reveal an increase significant of total protein and a decrease in the rate of carbohydrates. With respect to biomarkers, we note a significant decrease in the level of GSH. The results of this study demonstrate a metabolic imbalance associated oxidative stress in *Saccharomyces cerevisiae*.

Keywords: Saccharomyces cerevisiae; heavy metals; cadmium; biomarkers; pollution; GSH.

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1. INTRODUCTION

The problem of pollution is not a recent phenomenon, or accidental, it is oldest probably as old as the company itself human. Substances from any human activity (industrial, agricultural, ecological etc.) are likely to contaminate the environment in the short or medium term and are responsible of various alterations of the environment and its degradation (global warming, climate change, disruption of ecosystems and reduction of the ozone layer [1].

Contamination of the various environmental compartments by heavy metals is a hot topic. Research undertaken against this form of pollution show harmful on the biosphere and these effects at low levels. This contamination is not only the degradation of ecosystems with consequences on wildlife, flora and biodiversity but it also represents a real public health problem [1].

A significant amount of heavy metals is introduced into the environment through natural and human sources. This contamination has several origins such as: the burning of fossils, the exhaust of vehicles, incineration, mining, agriculture and solid and liquid waste. But it can also be naturally through the weathering and erosion of rocks, emissions from groundwater contaminated by rocks very responsible for the fires of forests as well as the volcanoes that release an average of natural in the world of 800 to 1400 tons of Cadmium entering the atmosphere [2]. The use agencies live bioindicators and bioaccumulator of pollution such as the yeast *Saccharomyces cerevisiae* is a necessary tool for measuring concentrations of pollutants but also measure the effects at the cellular and subcellular levels, allowing to serve in case of disruption of alarm signal [3].

Our work is a contribution to the behavior of *Saccharomyces cerevisiae* screw against a xenobiotic and more particularly a heavy metal: Cadmium. The effects of this heavy metal on cell growth, non-enzymatic biomarkers and especially energy metabolisms are studied.

2. MATERIALS AND METHODS

2.1 Biological Material

2.1.1 Choice of species

The biological material used in our study is a single-celled eukaryotic fungus; Yeast: *Saccharomyces cerevisiae*. Our choice fell on this fungus for multiple reasons:

- Yeasts are among the easiest microorganisms to grown as shown in the diversity of their agri-food application. Then, they are an excellent educational tool for acquiring control in main techniques of Microbiology [3].
- The unicellular state, the ability to multiply rapidly and the hardiness of the nutritional requirements gives these eukaryotes of the qualities that allow to study them and used easily as prokaryotes. Yeast can survive in aerobic conditions not applicable with higher organisms [3].

- Yeasts have a structure more complex than those of bacteria including the presence of a nucleus, mitochondria, Golgi and a chromosome apparatus. Because of their internal complexity and their enzyme content, they have a great capacity to transform various types of substrates in various products [3].

2.1.2 Classification

Baker's yeast is a unicellular fungus of the class Ascomycetes, genus Saccharomyces (the name refers to its affinity for sugar) and species *cerevisiae* (the name refers to its role in the production of beer); *Saccharomyces cerevisiae* the name was given to the yeast in 1838 by Meyer [4].

2.1.3 Medium composition

The biological material used is a microorganism unicellular eukaryote yeast *Saccharomyces cerevisiae*. The yeast is grown in the culture medium glucose yeast extract (20g glucose, 5g yeast extract and 1000 ml of distilled water, pH 5.6) [5].

2.1.4 The culture of yeasts

The yeast of industrial origin such as Baker's yeast is packaged in paste or lyophilized from culture in a medium rich in substrates. As a result, these substrates are often present in these preparations and breathing in yeast put in suspension in the water and / or ferment spontaneously. In these circumstances, it is difficult to carry out reliable measurements on the energy metabolism. Also, before any experience with measures on energy metabolism, it is necessary to thoroughly wash the yeast to remove all traces of substrate in the middle [6].

2.2 Chemical Material

The chemical material used is a heavy metal: Cadmium Chloride ($CdCl_2$), a stock solution of 10 mM is prepared; the weight concentration of the solution is 2.3 g / l of distilled water. Different dilutions of $CdCl_2$ represent the following concentrations: 1 mM, 5 mM and 10 mM, they are prepared from dilutions of the stock solution [3]. It is naturally rare in the environment where it is often found associated with zinc, but its industrial uses and thus its dissemination opportunities have largely increased since sixty years [7]. The chemistry of water-soluble cadmium is affected by many parameters such as pH or salinity Fig. 1.

2.3 Methods

2.3.1 Measured parameters

The extraction of metabolites was performed according to the method of Shibko et al. [9] with which the determination of carbohydrates is performed according to the method of Duchateau and Florkin [10], glutathione is estimated by a method of Weckberker and Cory [11], the proteins were quantified by the method of Bradford [12]. The Kinetics of growth of yeast is done by measuring the optical density (OD) at wave length λ= 620nm [6].

2.3.2 Statistical analysis

The results are shown as mean ± standard error, the results are compared by the nonparametric Kruskal-Wallis test, the software MINITAB Version 14.0, the significance level chosen is $p<0.05$ [13].

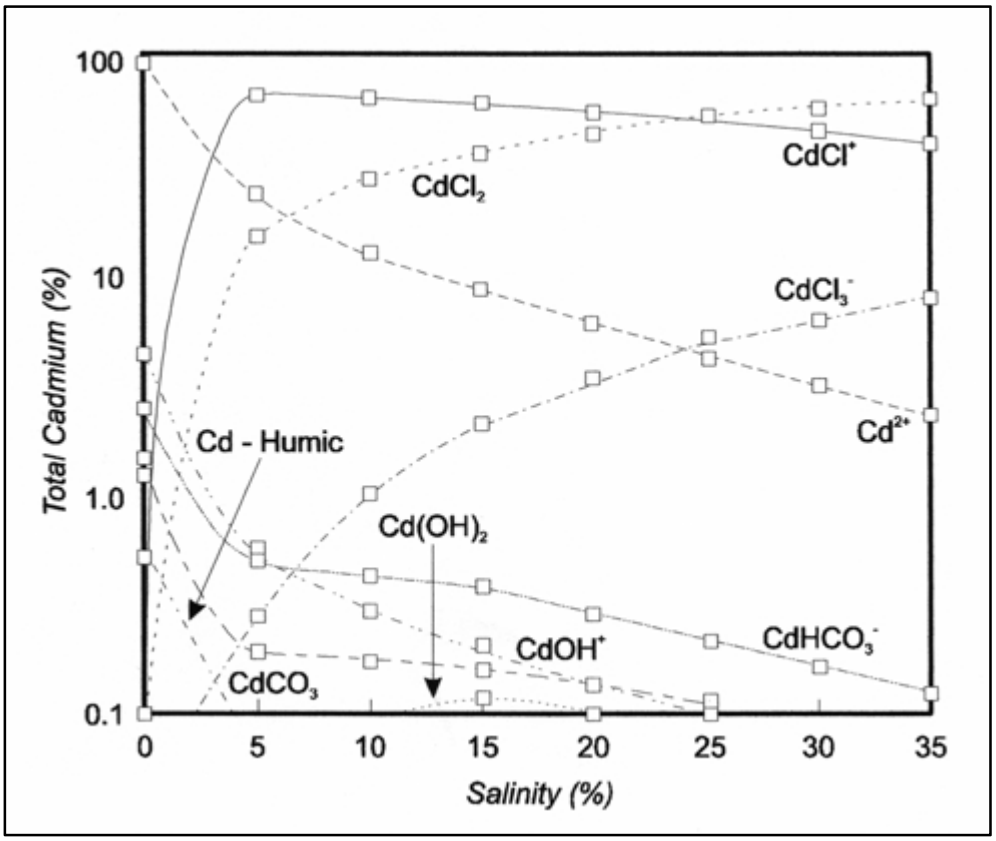

Fig. 1. Distribution of soluble chemical species of Cadmium depending on the salinity, pH 8 [8]

3. RESULTS

3.1 Effect of Cadmium on the Carbohydrates

Fig. 2 shows changes in the average levels of total carbohydrate obtained regarding the treatment of the yeasts with different concentrations of Cadmium. Statistical analysis shows a significant difference between the rate of carbohydrate in controls and the treated by the concentration (1 mM) with ($p = 0.04$), as well as a highly significant difference recorded in the treated by concentrations (5 mM) with ($p = 0.004$) and (10 mM) with ($p = 0.003$).

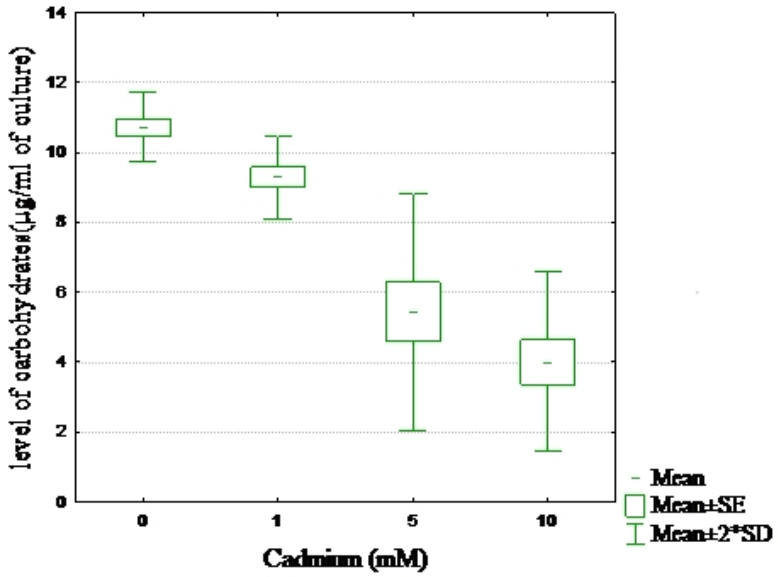

Fig. 2. Variations of carbohydrates levels in *Saccharomyces cerevisiae* under the effect of different concentrations of Cadmium

3.2 Effect of Cadmium on the Total Proteins

Fig. 3 shows variations in levels of average of the total protein in yeast controls and treated with different concentrations of Cadmium. Statistical analysis shows that there is no significant difference between the rate of proteins in witnesses and treaties by concentrations of cadmium: (1 mM) with (p = 0.676), (5 mM) with (p = 0.454) as well as a highly significant difference for concentration (10 mM) with (p = 0.009).

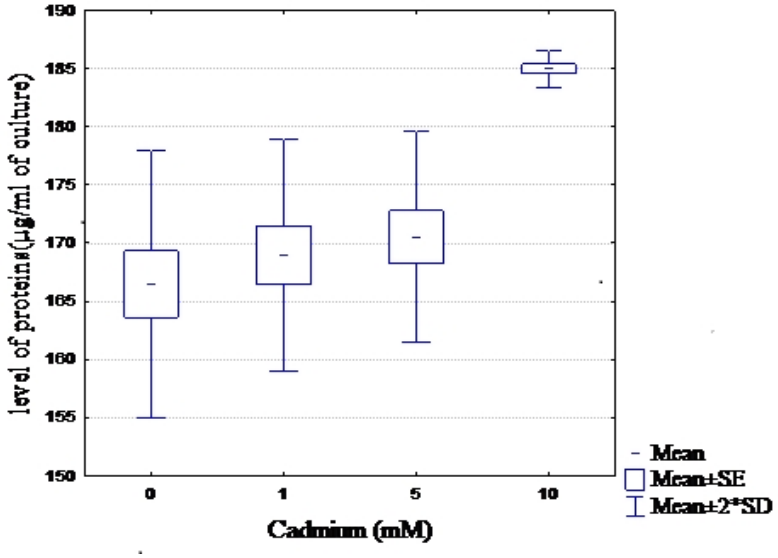

Fig. 3. Variations of proteins levels in *Saccharomyces cerevisiae* under the effect of different concentrations of Cadmium

3.3 Effect of Cadmium on Glutathione (GSH)

Fig. 4 shows changes in the rate of GSH obtained regarding the treatment of the yeasts with different concentrations of Cadmium. We note that in the presence of the xenobiotic (Cd) the level of GSH decreases in cells treated by the concentration (1 mM) in a highly significant manner with (p = 0.003) compared with controls. However this rate decreases in a way very highly significant (p = 0) in the processed by (10 mM) and (5 mM) concentrations compared with controls.

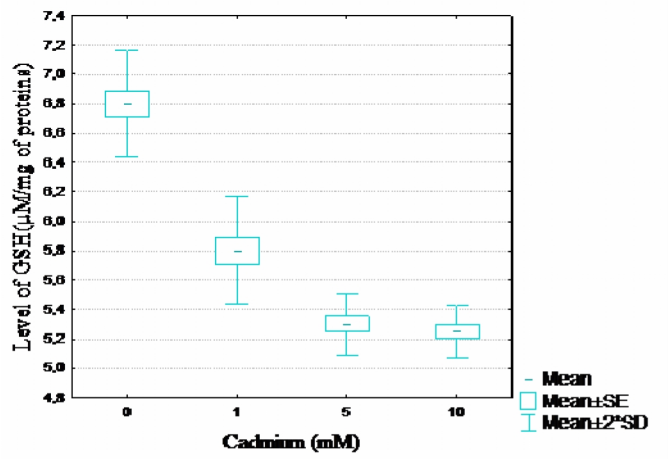

Fig. 4. Effect of Cadmium on GSH levels in *Saccharomyces cerevisiae*

3.4 Effect of Cadmium on the growth of *Saccharomyces cerevisiae*

According to Fig. 5, statistical analysis shows that there is a significant difference between control cells and treated by the concentration (1 mm) of cadmium with (p = 0.028). As well as a highly significant difference between control cells and treated by the (5 mm) concentration (p = 0.005). Also note a difference very highly significant between witnesses and those cells treated by the concentration (10 mm) with (p = 0).

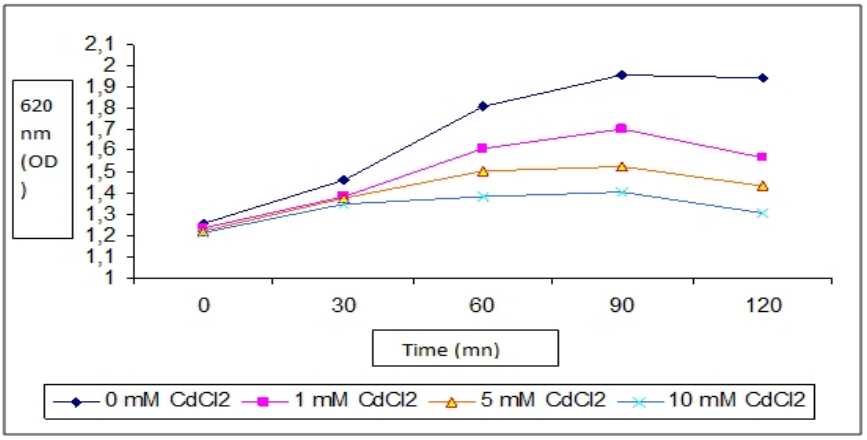

Fig. 5. Variations of growth in *Saccharomyces cerevisiae* under the effect of different concentrations of Cadmium

4. DISCUSSION

Cadmium is indeed a highly toxic metal that is widely present in industrial waste. However, the reasons for this toxicity are not well understood although its ability to react with the thiol groups of proteins has been suggested. A research team has combined structural studies by nuclear magnetic resonance and molecular dynamics experiments with biochemistry and genetics to describe, under physiological conditions, the early stages of the management of cadmium in the cell [14].

Numerous studies have shown that most metals are considered real agents toxic, disturbing some enzyme systems and also the metabolic and physiological activities in humans, animals and the plant [15].Metals generate radicals oxygen such as the powerful radical hydroxyl (OH) toxic at the cellular level that are at the origin of the phenomenon known under the more general term 'oxidative stress'. In yeast, heavy metals can induce a State of general stress, resulting in the reduction of their capacity adaptation to Anoxia [16].

The cytotoxic effects of a xenobiotic assessment can be carried out in using different parameters, among which: cell growth which reflects in microorganisms State of the metabolism of the cell [17].Thus our results show that Cadmium affects significantly the growth of *Saccharomyces cerevisiae*. Indeed (1 mM), it is close to those of control cells, (5 mm) it is reduced and from (10 mM) significant inhibition is noticed.

The tested xenobiotic is toxic to the yeast, this toxicity is manifested by an inhibition of cell growth, this brings us to confirm the influx of Xenobiotic inside cells, despite the presence of the cell membrane which constitutes a barrier against the massive entry of Xenobiotic but which nevertheless remains permeable [18]. These results are in agreement with those of Einicker-Lamas et al. [19] who have studied the toxicity of zinc and copper on *Euglena gracilis* (delicate chlorophyll algae). It is the work of Fukusshina et al. [20], which show that the Paramecium was a very close microorganism of higher organisms and that its growth was inhibited in the presence of certain heavy metals which makes it an excellent bioindicator of pollution. These same results are confirmed by Fenske and Gunther [21] and Khaldi [22].

On the principle that any type of chemical stress causes a release of free radicals in the body [23], an alteration of cellular components occurs when these phenomena intensity increases abnormally. All cellular components may be affected: protein, carbohydrates [24].

Our results highlight a significant increase in the rate of protein. These results are the same as those of Peccini et al. [25] and Masaya et al. [26] that highlight a significant increase in the rate of protein under the effects of a chemical stress in different biological models (ciliate protists, tadpole, alga. etc).Our hypothesis is that the increase in the rate of protein could be linked to triggers the process of detoxification put into play by the yeast cells. On the other hand, Fenske and Gunther [21] suggest an early induction of the synthesis of storage (MTs and granules) structures related to toxic kinetics. Addition of high concentrations of Cadmium, lead and zinc can induce dose-dependent synthesis of stress proteins in culture.

However, we noted that the rate of carbohydrate decreases generally dose-dependent in the presence of Cadmium, this decrease is due to the oxidation of carbohydrates in the presence of ions of Cadmium leading to the release of aldehyde and hydrogen peroxide. Carbohydrates are the primary sources and immediate energy, under conditions of

stress, carbohydrate supplies are depleted to meet the energy demands, these results are consistent with those of EL-Wakil and Radwan [27].

The antioxidant defense system is present in all aerobic cells and neutralizes the intermediate chemical reactions produced by endogenous pathway or metabolism of Xenobiotic [28].The activity of the antioxidant system may suffer an increase or an inhibition under the effects of a chemical stress. Glutathione is the major non-enzymatic antioxidant in all cells. It is reducing compound sulphur the most abundant in the intracellular compartment. By catching a hydroxyl radical, glutathione generates a superoxide radical, which must be supported by a SOD, in addition to its essential role of reducing agent; glutathione is involved also in a second level in anti-free radicals defense by his involvement in detoxification reactions catalyzed by glutathione-S-transferase. In our work; we highlighted a decrease very highly significant of GSH in the presence of Cadmium (5 mM and 10 mM), this depletion can be explained by the direct binding of glutathione to the metal [29,30].

On the other hand, the reactions of metals with glutathione are translated the formation of complexes [metal-GSH] or by the oxidation of GSH [31]. According to the study by Christie and Costa [32]; metals resulting in the oxidation of GSH are copper, manganese, iron and chrome while that stable complexes with GSH are formed by the zinc, cadmium, lead and nickel, and both these reactions could explain the decrease in glutathione. On the other hand, the reduction of the level of GSH can be explained also by the increase of use of the latter by the GST in the conjugation reaction [31].

5. CONCLUSION

This study is conducted to evaluate the effect of oxidative stress generated by a heavy metal: Cadmium, on an alternative cell model presented by a single-celled fungus: *Saccharomyces cerevisiae*. This crucial issue, to which we are interested, is summed up in the fact that the treatment of yeast by different concentrations of Cadmium affects growth and causes a decrease in glutathione levels, as well as a disruption of the average levels of the metabolites (carbohydrates and proteins).

In conclusion, it is clear that the species *Saccharomyces cerevisiae* is an excellent bioindicator of the degradation of the environment; it is sensitive to the presence of the Cadmium. Thus, it is undeniable that the use of yeasts is an indispensable tool for monitoring our environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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