Anti-oxidant activity research of enzymatic biomarkers in protozoa (*Paramecium sp.*) under stress by manufactured nanoparticles

Recherche de l'activité anti-oxydante des biomarqueurs enzymatiques chez les protozoaires (Paramecium sp.) sous un stress des nanoparticules manufacturés

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Abstract. The production of nanoparticles (NPs) is increasing rapidly for electronics, chemistry and biology applications. This interest is due to the very small size of NPs which provides them with many interesting properties such as rapid diffusion, high specific surface areas, reactivity in liquid or gas phase and a size close to bio-macromolecules. However, these extreme abilities might be a problem when considering a potentially uncontrolled exposure to the environment. For instance, nanoparticles might be highly mobile and rapidly transported in the environment or inside the body through a water or air pathway. Accordingly, the very fast development of these new synthetic nanomaterials raises questions about their impact on the environment and human health. We have studied the impact of manufactured nanoparticles (ZnO) on a ciliated microorganisms (*Paramecium sp.*). Principles results show that the ZnO presence affects the paramecia growth. On the metabolic level, ZnO causes a disturbance in the rate of total protein and total carbohydrates. Regarding the biomarkers we identified disruption of glutathione (GSH) and catalase (CAT) activity. This study shows the importance of a model species for assessing the ZnO toxicity in laboratory tests. It is clear that ZnO is the oxidative stress source, which results in a stimulation of growth in *Paramecium sp*., a disturbance in the total protein rate, carbohydrates, glutathione and catalase activity.

Keywords: Biomarkers, Catalase, Cell growth, GSH, Metabolites, Nanoparticles, ZnO, *Paramecium sp.*

Résumé. La production des nanoparticules (NP) augmente rapidement pour des applications dans les domaines d'électronique, la chimie et la biologie. Cet intérêt est dû à la très petite taille des NPs, qui leur fournit de nombreuses propriétés intéressantes telles que: la diffusion rapide, des surfaces spécifiques élevées, la réactivité en phase liquide ou de gaz et d'une taille proche de bio-macromolécules. Ces capacités extrêmes pourraient être un problème lors de l'examen d'une exposition potentiellement incontrôlée dans l'environnement. Par exemple, les nanoparticules peuvent être très mobiles et transportés rapidement dans l'environnement ou à l'intérieur du corps par une voie d'eau ou de l'air. En conséquence, le développement très rapide de ces nouveaux nanomatériaux synthétiques soulève des questions quant à leur impact sur l'environnement et la santé humaine. Nous avons étudié l'impact des nanoparticules manufacturées (ZnO) sur un micro-organisme cilié (*Paramecium sp.*). Les principaux résultats montrent que la présence de ZnO affecte la croissance des paramécies. Sur le plan métabolique, ZnO provoque une perturbation du taux de protéines totales et glucides totaux. En ce qui concerne les biomarqueurs, nous avons identifié la perturbation du glutathion (GSH) et de l'activité catalase (CAT). Cette étude montre l'importance d'une espèce modèle pour évaluer la toxicité de ZnO dans des tests de laboratoire. Il est clair que ZnO est la source de stress oxydatif, qui se traduit par une stimulation de la croissance chez *Paramecium sp.,* une perturbation du taux des protéines totales, des hydrates de carbone, du glutathion et de l'activité catalase.

Mots-clés: Biomarqueurs, Catalase, Croissance cellulaire, GSH, Métabolites, Nanoparticules, ZnO, *Paramecium sp.*

INTRODUCTION

Numerous studies have shown that the metal nanoparticles toxicity involves a dissolution of nanoparticles and therefore chemical toxicity associated with ions released. The ZnO metal nanoparticle toxicity is always compared to Zn^{2+} ions and generation of Reactive Oxygen Species (ROS) as the major mechanism involved in the nanoparticles cellular toxicity (NPs) (Oberdorster *et al.* 2005, Unfried *et al.* 2007).

Microorganisms and especially ciliates are widely used as models to study cellular mechanisms of xenobiotics action and as pollution bio-indicators (Radix *et al.* 2000). In this study, we set ourselves the aim to highlight the effect of a xenobiotic (metal nanoparticle ZnO) on newly synthesized ciliates of freshwater paramecia (Fig. 1). These microorganisms are considered with other protists (*Tetrahymena*) as a good alternative model for toxicological studies. Evaluation of cytotoxic effects of nanoparticles (NPs) metal (ZnO) can be achieved by using different parameters, including cell growth in microorganisms which reflects the cell metabolism state. The nanoparticles biological effects study promises to be a cumbersome and complex discipline, due to the large number of existing nanoparticles, but also the features large number (size, shape, surface, functionalization ...) can influence their biological effects. And *in vitro* studies seem to be a necessary first step as particularly suitable for testing a large number of conditions (Auffan 2007).

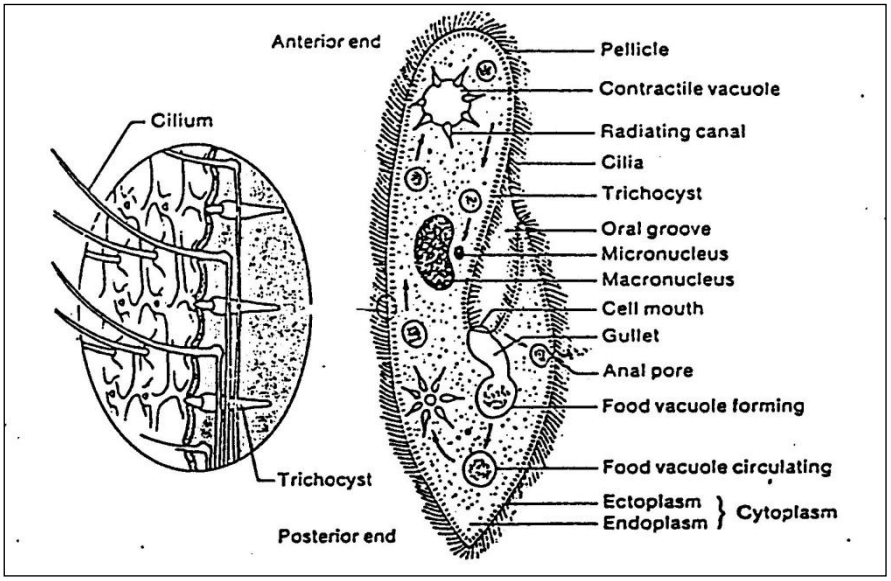

Figure1. General appearance of a paramecium (Beaumont & Cassier 1998).

They do not absolve course of studies in *vivo*, but have the advantage of establishing a first selection of nanoparticles having probably an effect on humans and the environment. Due their recognized catalytic activity, the hypothesis of generation Reactive Oxygen Species (ROS) was first put forward (Auffan 2007). Action and consequence mechanisms, cellular and molecular level of exposure to metal oxide nanoparticles seem to depend on many parameters and need to be clarified. The biological effects of nanoparticles depend on their characteristics, but it seems that the nature is the source of their reactivity and hence their interaction with the living organisms. Thus, in the same way as for chemicals, it seems more appropriate to treat nanoparticles biological effects ~~of~~ according to their nature to elucidate the implications and risks of nanoparticles on ecosystems and human bodies in particular (Angelique 2008).

MATERIALS AND METHODS

Crystallographic characterization by X-Ray Diffraction "XRD"

Figure 2 shows the X-ray diffraction pattern of ZnO. We note that the powder has good characteristic peaks of wurtziteh exagonal structure corresponding to the diffraction planes of the ZnO wurtzite structure. The diffractograms has a good crystallin quality of the developed NPs (Bouloudenine 2006). For our structure we calculated the parameters and the volume of the unit cell. Crystallites constituting our powder have 59.10nm as a mean size calculated by the Debye Scherrer formula. The results of X-ray diffraction show that the synthesized powder has good diffraction lines of the ZnO wurtzite structure "a=3.24982(9), c =5.20661(15)" without the occurrence of spurious phases (Bouloudenine 2006).

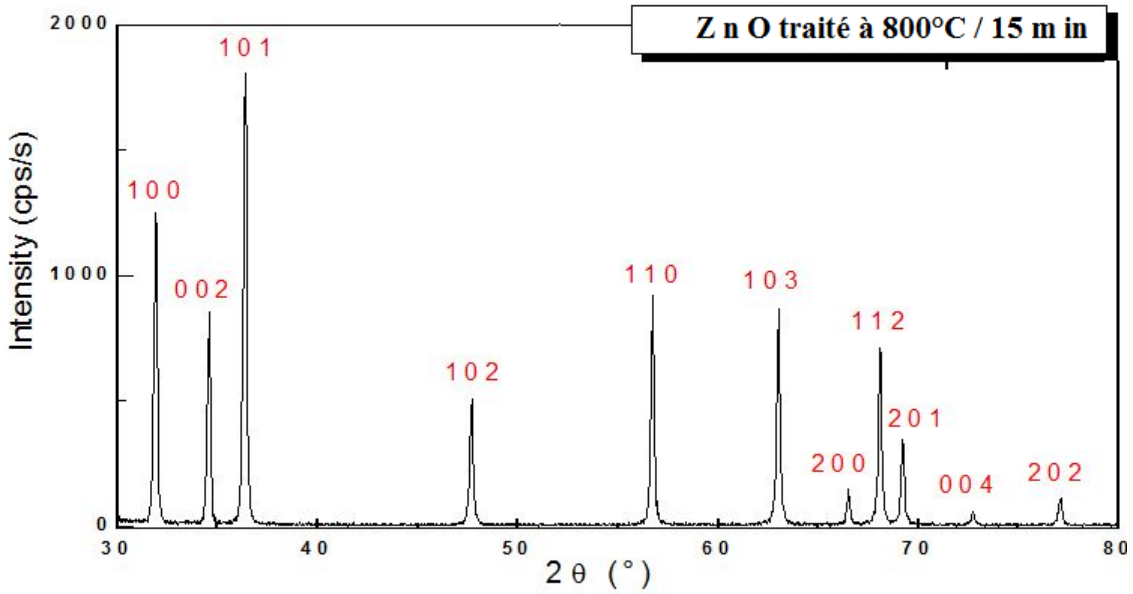

Figure 2. Ray "X" diffraction pattern of ZnO powder, CoKα1 (λ =1.789A°) (Bouloudenine 2006).

Transmission Electron Microscopy "TEM"

Figures 3 a and b show "TEM" images of polycrystallin ZnO powder. At low resolution TEM image (a), we observe ZnO crystallites with hexagonal shapes in 100 nm average sizes. High resolution images (b) reveal a well-crystallized structure which crystallographic planes are parallel to the direction (100) (Bouloudenine 2006).

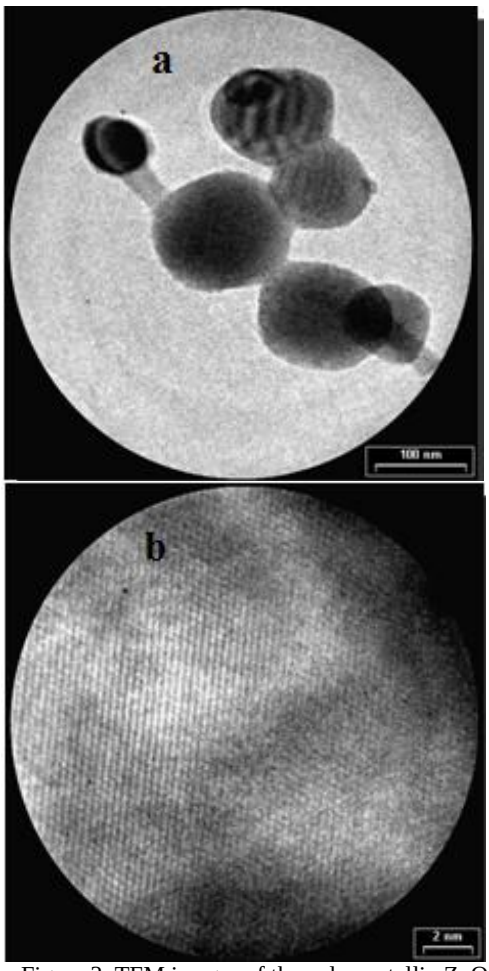

Figure 3. TEM images of the polycrystallin ZnO powder (a, b) (Bouloudenine 2006).

Heat treatment

After several attempts, temperature varying between 400 °C and 800 °C, we chose to treat the powder at 800° C because this condition allows obtaining well-crystallized and singling grains (Bouloudenine 2006).

The procedure involves placing a white powder (alumina crucibles) for 15 minutes in a kiln programmed to 800 °C, then take them out quickly (quenching). The possibility of unwanted oxides formation is limited by the short time heat treatment. Upon exiting the oven we get a yellow powder, powder obtained is crushed before characterization (Bouloudenine 2006).

Mixed Cultures

In a container of rain water (drinking water is often bleach) we infused macerate hay, filtered after 24 hours and let the infusion (filtrate) at 15° C in a dark place, with neutral pH and well ventilated (do not cover the container) (Beaumont & Cassier 1998). This work was carried out during 3 months from March because cultivation and growth of the *paramecium* requires an ambient temperature of the environment.

Growth measurement

The growth kinetics of paramecium has been carried out by measuring the optical density (OD at 600 nm (nanometer) taking the wavelength as a function of time (Lavergne 1985). Different treatments of zinc oxide are carried out in culture media and growth kinetics is monitored as a function of time for both witnesses of treatment.

Determination of total protein and carbohydrate

The proteins are assayed by the method of Bradford (1976) using Albumin of Beef Serum (BSA) as standard. The calibration range is carried out from a stock solution of BSA (1 mg/ml). The protein assay is carried out with a 100 µl aliquot. The optical densities are measured by a spectrophotometer (JENWAY 63000), the measurement is carried out at a wavelength of 595 mm. Total proteins are determined from the reference curve. Total carbohydrate were determined by the method of Duchateau & Florkin (1959) using anthrone in sulfuric acid.

Determination of biomarkers

The glutathione was assayed by the method of Weckberker & Cory (1988) based on measuring the absorbance of the 2-nitro-5mercapturic resulting from the reduction of the 5-5 thiol-bis-2-nitrobenzoic acid (DTNB) by the thiol groups (-SH) glutatathion. We use for measuring the activity of catalase (CAT) the method of Regoli & Principato (1995). The absorbance decay is recorded for three minutes by a spectrophotometer (JENWAY 6300) for a wavelength of 240 nm and a molar extinction coefficient ε = 39400 M^{-1} cm^{-1}. For a final volume of 3 ml, the reaction mixture contains: 100 µl of the crude enzymatic extract, 50 µl of 0.3% hydrogen peroxide H_2O_2 and 2850 µl of phosphate buffer (50 mM, pH=7.2). The calibration of the apparatus takes place in the absence of the enzymatic extract. The reaction is initiated by the addition of hydrogen peroxide. The catalase activity is expressed in nmol/min/mg of proteins.

Statistical Analysis

The statistical analysis is performed by the test (T) who is used to compare two samples (control and treated). This test is performed using the data analysis software: Statistica (Dagnelie 1999).

Table 1. Experimental conditions of synthesis (mol/l: Mole per liter; g/mol: grams per liter; ml: milliliter).

Reagent	Concentration (mol/l)	Molar mass (g/mol)	Volume of solvant (ml)
$[Zn(CH_3COO)_2, 2H_2O]$	0,4	219.48	50
$[C_2H_2O_4, 2H_2O]$	0,4	126.07	50

RESULTS AND DISCUSSION

In this section, we present the results of our work related to the measured parameters. We opted initially for a test of cytotoxicity to classify the toxicity of these molecules (ZnO metal nanoparticle) tested through the evolution of the curve growth of *paramecium* culture (Tab. 2). Paramecia cells are resistant to damage by a metal nanoparticle ZnO tested in our work. It is in agreement with Unfried *et al.* 2007 work, which began to show a resistance of *C. metallidurans.* CH_{34} is much stronger after penetration of NPs nanoparticles (titanium oxide, TiO_2) and aluminum oxide (Al_2O_3) of the bacterial cell wall. The tested xenobiotic acted as a physiological molecule involved in the major metabolic pathways (growths paramecia). Our results are in agreement with those of Kindermann *et al*. (2005), which demonstrated that this effect is due to Zinc released after ZnO dissolution. It is a physiological metal which is involved in the major metabolic pathways either as a cofactor or as a component of the enzymes structure such as the case of Vander Oost *et al*. (2003). Other studies including Fang *et al.* (2007) confirm our results and show a variation in the growth of the lipid composition.

Similarly, according Tsao *et al*. (2002), carboxy fullerènes and cationic derivatives of fullerenes would integrate itself to the wall which would cause a destabilization of the membrane and thus cellular functions as has been shown for the chain respiratory present in the membrane (Mashino *et al*. 1999, Mashino *et al*. 2003). In the same way, other studies (including Fang *et al.* 2007) confirm our results, showed a change in the membrane lipid composition in the presence of fullerenes, which is an evidence destabilization and/or an adaptation of the bacteria. In the case of destabilization, this could be due to the formation of reactive oxygen species that training for example peroxidation of membrane lipids (Beaumont & Cassier 1998). Due their surface reactivity, nanoparticles are capable inducing the production of reactive oxygen species (ROS) and cause oxidative stress to the cell (Foucaud *et al*. 2007, Garza *et al*. 2008, Long *et al*. 2006). With the principle that any type of chemical stress causes oxidative damage and can cause oxidative stress and significantly affect cellular function. During oxidative stress, ROS not detoxified by the antioxidant system will oxidize macromolecules, such as lipids, proteins, sugars and nucleic acids, disrupting their chemical structures and altering their biological functions (Igor 2011). In addition, general or localized excess zinc is responsible for its toxic effects (Plum *et al*. 2010). Zn can move from an antioxidant to a prooxidant role, causing the formation of free radicals indirect (Igor 2011). Our results show a disturbance in the rate of total protein (Fig. 4) and carbohydrate (Fig. 5) under the effect of chemical stress in both biological models used.

Our hypothesis is that the increase in protein levels, 0.25 and 1mM of ZnO (Fig. 4) could be explained by induction of detoxification processs in motion by paramecium. The decrease in total protein levels (2.5 mM of ZnO) observed at the end of treatment is explained biochemically by several mechanisms including lipoproteins formation which are used to repair damaged cells and tissue or direct use by cells for energy needs in these stress conditions (Radwan *et al*. 2008). These results are in agreement with Padmaja & Rao (1994), which showed a decrease in protein level in the digestive gland of the snail fresh water, *Bellamya dissimilis*, after exposure to pesticides (Carbamates).

Further disruption rate of total carbohydrate (Fig. 5) observed in paramecia are explained by the depletion of energy requirements (carbohydrates). These results are consistent with those of El-Wakil & Radwan (1991), who suggested that glycogen exhaustion content in the tissue of the fresh water snail, *Bellamya dissimilis,* exposed to Endosulfan, parathion methyl, and quinal-phosof-Nuvan (pesticides) would result from the use of glycogen for the generation energy; the request is due to the induction of hypoxia caused by pesticides.

On the other hand, reduction of GSH (Fig. 6) can also be explained by the increase in its use with the GST (Glutathione-S-Transferase) in the conjugation reaction (Canesi & Viarengo 1997, Regoli *et al*. 1998). The glutathion (GSH) scavenges reactive oxygen species because it reacts especially with the hydroxyl radical (OH) and O_2 (Saez *et al*. 1990). Canesi *et al*. (1999) found a decrease of glutathione in the digestive gland of the mussel (*Mytilusgallo provincialis*) after 3 days of exposure to copper. While Wanget *et al*. (2008) have found a dose-dependent on the rate of glutathione in hepato-pancreas of fresh water crabs exposed to higher concentrations of cadmium.

Our results showed an increase in catalase activity (0.25 and 2.5 mM, Fig. 7) in these ciliates at the presence of ZnO, probably due to increased antioxidant activity in *Paramecium* cells, according to Halliwell & Gutteridge (1985), the increased oxidative stress enhances the activity of antioxidant enzymes. Indeed, Catalase activity consists of an hydrogen peroxide (H_2O_2) transformation in water 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 metals (Vander Oost *et al*. 2003, Brown *et al*. 2004), derived reactive oxygen can lead to oxidation of macromolecules (DNA, lipids and proteins) (Vlahogianni *et al*. 2007).

Table 2. Optical Density of the Effect of ZnO on the cell growth of *paramecium* function of time (Time = days, n=5); 0mM=Control; ±: standard error of the mean values; ZnO = nanoparticles; OD: Optical Density; mM = milliMolair (10-3Mole).

Days	1	2	3	4	5	6	7	8	9	10	11
Exposure time (h)	**24**	**48**	**72**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**288**
0mM ZnO	0.114±0.09	0.125±1.03	0.118±0.99	0.180±0.78	0.150±0.84	0.145±1.08	0.254±0.67	0.476±0.88	0.295±0.45	0.303±0.23	0.354±0.44
0.25mM ZnO	0.189±0.77	0.345±0.89	0.190±0.72	0.424±0.56	0.175±0.69	0.155±0.88	0.390±0.79	0.230±1.09	0.570±0.45	0.524±0.84	0.702±0.62
1mM ZnO	0.250±0.19	0.125±0.59	0.160±0.87	0.224±0.96	0.168±0.91	0.142±1.10	0.327±0.58	0.512±0.81	0.409±0.62	0.649±1.01	0.627±0.31
2.5mM ZnO	0.526±0.90	0.138±0.50	0.184±0.63	0.153±0.71	0.165±0.68	0.190±0.19	0.171±1.12	0.258±1.05	0.330±0.86	0.390±0.29	0.691±1.21

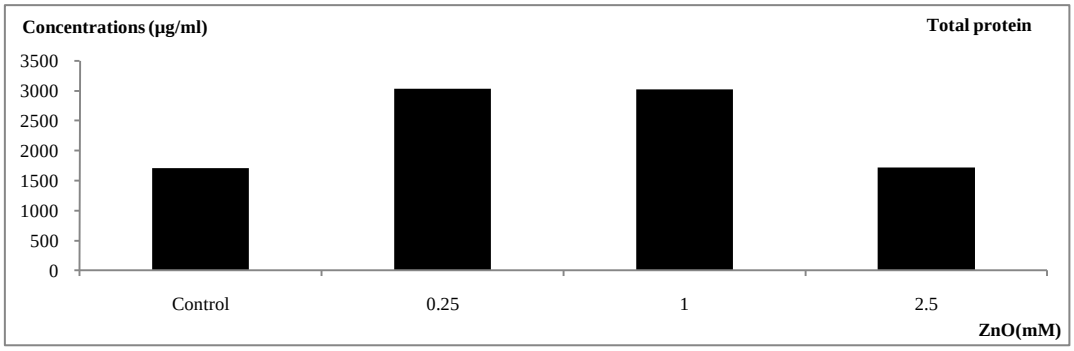

Figure 4. Effect of ZnO on changes in mean levels of total protein (n=5) (µg/ml: micrograms per milliliter of culture medium of *paramecium).*

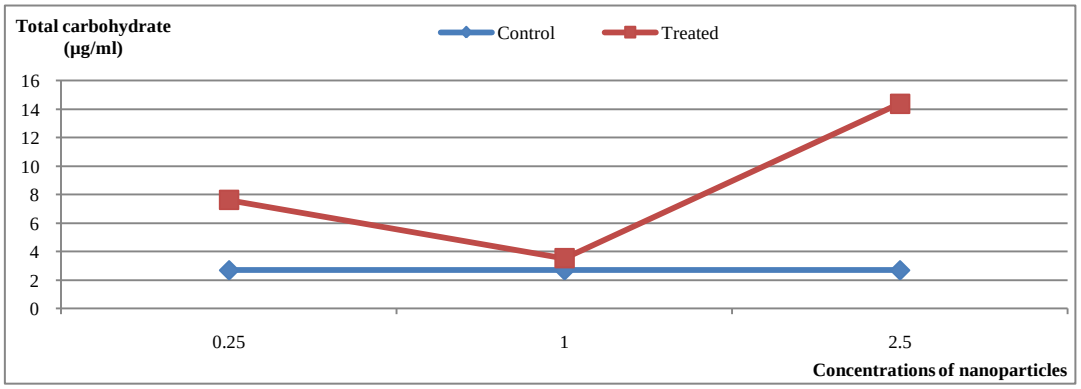

Figure 5**.** Effect of ZnO on changes in mean levels of total carboyhdrate.

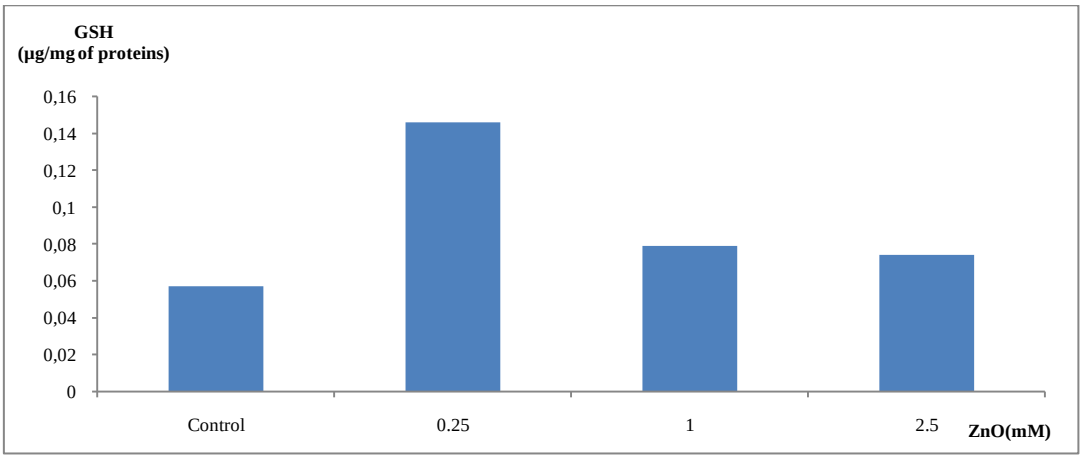

Figure 6. Impact of different concentrations of nanoparticles on glutathione (GSH) in *Paramecium sp*.

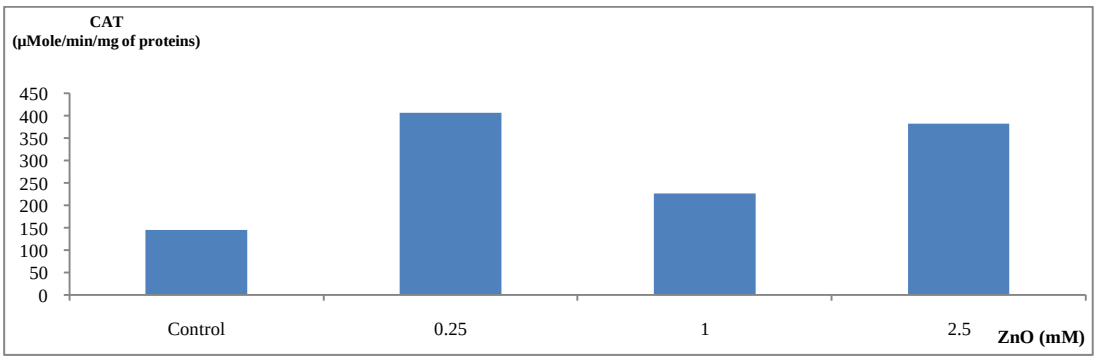

Figure 7. Evolution of catalase (CAT) enzyme activity according to the different concentrations of nanoparticles in *Paramecium sp.*

CONCLUSION

1. This *in vitro* study shows the interest of this biological model (Paramecium) for assessing the Zn0 toxicity (manufactured nanoparticles) in tests laboratory.
2. It seems clear that this xenobiotic is the source of : (i) oxidative stresses that resulting in stimulation of growth in *Paramecium sp,* (ii) disturbance levels of total protein and carbohydrates, glutathione and activity catalase.
3. This confirms that the uses of paramecia are essential to monitor our environment.

REFERENCES

Angelique S.D. 2008. *Effets biologiques des nanoparticules manufacturées : influence de leurs caractéristiques*. Thèse de Doctorat. Institut des Sciences et Industries du Vivant et de l'Environnement (Agro Paris Tech), 140 p.

Auffan M. 2007. *Nanoparticules d'oxydes métalliques : Relations entre la réactivité de surface et des réponses biologiques*. Thèse de Doctorat University of Paul Cézanne AIX-Marseille III, France, 111 p.

Beaumont A. & Cassier P. 1998. *Travaux pratique de Biologie Animale, Zoologie, Embryologie et Histologie*. 2ème cycle. Capes. Agrègation. Editions Dunod, 123-143.

Bouloudenine M. .2006 . *Elaboration et caractérisation des semi-conducteurs magnétiques dilués de type Zn $_{1-x}$ Co_xO par voies chimiques* . Thèse de Doctorat en Physique des semi-conducteurs. Univ. Badji Mokhtar, Annaba.186 p.

Bradford M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the Principe of protein-dye binding. *Analytical Biochemistry*, 72, 278-254.

Brown P.J., Long S.M., Spurgeon D.J. *et al.* 2004. Toxicological and biochemical responses of the earthworm Lumbricus rubellus to pyrene a non-carcinogenic polycyclic aromatic hydrocarbon. *Chemosphere*, 57, 1675–1681.

Canesi L. & Viarengo A. 1997. Age-related differences in glutathione metabolism in mussel tissues. *Comparatif Biochemistry and Physiology*, 116, 217–221.

Canesi L., Viarengo A., Leonzio C. *et al*. 1999. Heavy metals and glutathione metabolism in mussel tissues. *Aquatic Toxicology*, 46, 67-76.

Dagnelie, P. 1999. *Statistiques théoriques et appliquées Tome 2. Références statistiques à une et à deux dimensions.* Bruxelles. Université de De Boeck & Larcier ed., 659 p.

Duchateau G. & Florkin.A. 1959. For treahalosemie of insects and its signification. *Archives of Insect Biochemistry and Physioogy*, 67, 306-314.

EL-Wakil H.B & Radwan M.A .1991. Biochemical studies on the terrestrial snail, Eobania vermicular treated with some pesticides. *Journal of Environment Sciences and health*, B20, 479-489.

Fang J., Lyon D. Y., Wiesner M. R. *et al.* 2007. Effect of a fullerene water suspension on bacterial phospholipids and membrane phase behavior. *Environment Sciences and Technology*, 41, 7, 2636–2642.

Foucaud L., Wilson M. R., Brown D. M. *et al.* 2007. Measurement of reactive species production by nanoparticles prepared in biologically relevant media. *Toxicology Letters*, 174, 1-3,1–9.

Garza K. M., Soto K. F. & Murr L. E .2008. Cytotoxicity and reactive oxygen species generation from aggregated carbon and carbonaceous nanoparticulate materials. *International Journal of Nanomedicine* 3, 1, 83–94.

Halliwell B., Gutteridge J.M.C. 1985. *Free Radicals in Biology and Medicine*. Oxford-University Press, New York. 346 p.

Igor P. 2011. *Étude in vitro de la toxicité de nanoparticules métalliques (TiO_2, ZnO, CdS) sur la cible rénale.* Thèse Doctorat, Université de Bordeaux Segalen, France, 118 p.

Kindermann B., Döring F., Budczies J. *et al.*. 2005. Zinc-sensitive genes as potential new target genes of the metal transcription factor-1 (MTF-1). *Biochemistry and cell biology*, 83, 221-229.

Lavergne M.1985 . *Etude des effets du Diazépam et du Medazepam sur un protiste cilié*. Thèse de Doctorat, Univ. Paris VII, 146 p.

Long T. C., Saleh N., Tilton R. D. *et al.* 2006. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2) : implications for nanoparticle neurotoxicity. *Environment Sciences and Technology.*, 40, 14, 4346–4352.

Mashino T., Okuda K., Hirota T. et al. 1999. Inhibition of *E. coli* growth by fullerene derivatives and inhibition mechanism. *Bioorganic and Medicinal Chemistry Letters*, 9, 20, 2959–2962.

Mashino T., Usui N., Okuda K. *et al.* 2003. Respiratory chain inhibition by fullerene derivatives: hydrogen peroxide production caused by fullerene derivatives and a respiratory chain system. *Bioorganic and Medicinal Chemistry Letters*, 11, 7, 1433–1438.

Oberdorster G., Oberdorster E., & Oberdorster J. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*, 113, 823-839.

Padmaja M.B. & Rao. 1994. Effect of an organochlorine and three organophosphate pesticides on glucose, glycogen lipid and protein contents in tissues of the freshwater snail, *Bellamya dissimillis* (Muller), *Bulletin of Environment Contamination and Toxicology*, 53, 142-148.

Plum L. M., Rink L., & Haase H. 2010. The essential toxin: impact of zinc on human health. *International Journal of Environmental Research and Public Health*, 7, 1342-1365.

Regoli F. & Principato G.1995. Glutathione, glutathione-dependant et antioxidant enzymes in mussel Mytilus galloprovincialis exposed to metals under field et laboratory conditions: implication for the biomarkers .*Aquatic Toxicology.* 31, 143-164.

Tsao N., Luh T.-Y., Chou C.-K. *et al.* 2002. *In vitro* action of carboxyfullerene. *Journal of Antimicrobiology and Chemotherapy*, 49, 4, 641–649.

Radwan M.A., Essawy A.E., Abdelmeguied N.E. *et al.* 2008. Biochemical and histochemical studies on the digestive gland of *Eobania vermiculata* snails treated with carbamate pesticides. *Pesticide Biochemistry and Physiology*, 90, 154–167

Radix P., Leonard M., Papantoniou C., Roman G. *et al*. 2000. Comparaison of four chronic toxicity tests using algae bacteria and invertebrates assessed with sixteen chemicals. *Ecotoxicology and Environmental Safety* 47, 186-194.

Regoli F., Nigro M., and Orlando E. 1998. Lysosomal and antioxidant responses to metals in the antarctic scallop *Adamussium colbecki*. *Aquatic Toxicology*, 40: 375-392.

Saez G.T., Bannister W.H. & Bannister J.V. 1990. Free radicals and thiolcompounds: the role of glutathione against free radical toxicity. *In:* Vina J. (Ed.), *Glutathione Metabolism and Physiological Functions*. *CRC Press, Boca Raton*, 237–254.

Unfried K., Albrecht C. Klotz L.O *et al.* 2007. Cellular responses to nanoparticles: Target structures and mechanisms. *Nanotoxicology*, 1, 52-71.

Vander Oost R., Beyer J. & Vermeulen N.P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environment Toxicology and Pharmacology*, 13, 57–149.

Vlahogianni T., Dassenakis M., Scoullos M.J. & Valavanidis A .2007. Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals pollution in coastal areas From the Saronikos Gulf of Greece. *Marine Pollution Bulletin*, 54, 1361-1371.

Wang L., Yan B., Liu N. *et al*.. 2008. Effects of cadmium on glutathione synthesis in hepatopancreas of freshwater crab Sinopotamon yangtsekiense. *Chemosphere*. 74, 1, 51-6. DOI : 10.1016/j.chemosphere.2008.09.025

Weckberker G. & G. Cory. 1988. Ribonucleotide reductase activity and growth of glutathione depleted mouse leukemial 1210 cells *in vitro*. *Cancer letters*, 40, 257-264.

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