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CYTOTOXIC EFFECT OF SNO_2 NANOPARTICLES ON ALTERNATIVE CELLULAR MODEL: PARAMECIUM TETRAURELIA

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ABSTRACT: The development of nanotechnologies and their uses, leads to an increase of nanoparticles concentrations in the air, water and soil. For better understanding the potential impacts of metal oxide nanoparticles in the ecosystem, the present study investigates the cytotoxic effect of SnO_2 nanoparticles for different grain sizes on the alternative model of freshwater, water pollution bioindicator, *Paramecium tetraurelia*. The size of SnO_2 nanometric powder (Sigma-Aldrich 99.99% pure) has been reduced using mechanical milling with different times. Obtained nanomaterials were then characterized by X-ray diffraction (XRD) and spectroscopy infrared Fourier transforms (FTIR). Moreover, the toxicity of SnO_2 nanoparticles on paramecium was studied by following the evolution of the growth kinetics and percent response as a function of time; whereas the impact of SnO_2 nanoparticles on paramecium was determined on two biomarkers of oxidative stress respectively, Catalase (CAT) and glutathione S-transferase (GST). The preliminary results show a non-negligible effect of SnO_2 (NPs) via their grain sizes. Indeed, it was recorded an increase in the number of paramecium at low concentrations of SnO_2 and its inhibition at high concentrations.

KEYWORDS: Nanoparticles, SnO_2, grain size, *Paramecium tetraurelia*, cytotoxicity.

INTRODUCTION:

Nanotechnology uses materials of which a single unit is sized between 1 and 100 nm (Mallakpour *et al.*, 2015). Due to its interesting chemical and physical properties, nanomaterials have attracted the attention of researchers around the globe. Nowadays, different products utilize various types of nanomaterials with variety of structures and properties including cosmetics (Matranga *et al.*, 2012), fungicides in agriculture, food industry (Martineau *et al.*, 2014) and electronic devices (Zhang *et al.*, 2011) etc. Transition metal oxides nanoparticles play a crucial role in photocatalysis applications (He *et al.*, 2013) and because of their nano-scale size, high aspect ratio (i.e. surface to volume ratio of atoms) and edge effect (Jan *et al.*, 2014) they exhibit desired properties which found to be applicable in many fields such as lithium-ion batteries (Subramanyam *et al.*, 2014), industry and medicine (Gambardella *et al.*, 2014), photodetectors and solar cells (Jan *et al.*, 2014).

Among these transition metal oxides, Tin Dioxide (SnO_2) is an important n-type semiconductor material (Meena Kumari *et al.*, 2015) with a wide band-gap (Eg =3.6 eV) (Suvetha Rani, *et al.*, 2013).A wide band gap of SnO_2 has attracted considerable attention wherever doped with transition metals owing to their remarkable electronic optical and magnetic properties resulting from large Sp-d exchange interaction between the magnetic ions and the band electron (Kaur *et al.*, 2012). It is extensively used in variety of applications such as; Catalysis (Karunakaran *et al.*, 2013), biomedical and biological sensing (Liu *et al.*, 2014), lithium batteries (Vidhu *et al.*, 2015), solar cells (Henry *et al.*, 2015), gas sensors, transparent conducting electrode (Vidhu *et al.*, 2015), and optical material (Fakhri *et al.*, 2015).

Many methods have been developed to synthesize SnO_2 nanostructure such as hydrothermal methods, sol-gel methods (Bhattacharjee *et al.*, 2015), microwave heating (Bhattacharjee *et al.*, 2015), chemical vapor deposition, co-precipitation, mechanical synthesis, laser pyrolysis and thermal evaporation (Bhattacharjee *et al.*, 2015).

Despite the significant contribution of nanoparticles in solving many of our daily life problems through their technological applications, the use of nanoparticles nowadays in many commercial products without clear studies on their effects on human and environment health raised many concerns. Thus, the utility of nanomaterials and nanoparticles is highly dependent on their toxicity. Alternative models are used in toxicology to understand the mechanisms of toxic actions at different levels of the organization of the biological cell.

Among these models, the *Paramecium tetraurelia* is a suitable one to investigate the cytotoxicity of SnO_2 NPs because it is already been used to study the effect of chemicals on aquatic community (Amamra *et al.*, 2015; Djekoun *et al.*, 2015).

The objective of our work is to study the toxicity effects of SnO_2 nanoparticles in the ecosystem. For this purpose, the toxicity of SnO_2 NPs was investigated by different grain size using the alternative model *Paramecium tetraurelia* to define their mechanisms of action and subsequently explain their toxicity.

MATERIALS AND METHODS:

Samples Preparation (ball milling)

Two grams of commercial SnO_2 powder (Sigma–Aldrich, 99.9% purity) were used as starting materials and milled during different times (0, 1and 3h) at room temperature under argon atmosphere, by using a

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planetary ball milling machine. Milling was performed using stainless steel balls with a diameter of 12.7 mm, and was loaded with 12.5 g. The relationship of ball-to-powder percent was equal to 97:3 and the rotation speed as maintained at 200 rpm.

Characterization Techniques

Ball-milled powders were characterized by X-ray powder diffraction (XRD) using X'Pert PRO PANalytical system with Cu radiation at wavelength λ =1.5405980 Å at 2θ values between 20° and 90°. The SnO_2 samples were analyzed by infrared spectrometry (FTIR), (*SHIMADZU FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER (FTIR-8400S)*. Two milligrams of the sample was mixed with 200 mg KBr (FTIR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FTIR spectra were recorded in the range 4000- 400 cm^{-1} in FTIR spectroscopy at a resolution of 4 cm^{-1}.

Cell Culture

The biological material used in our study is a ciliated protist: *Paramecium tetraurelia* (Azzouz *et al.*, 2011), kindly provided by Laboratory of Cellular Toxicology at the University of Annaba (Algeria).

Measurement of Growth Kinetics

The experiments were performed according to (Azzouz *et al.*, 2011).The SnO_2 NPs were dispersed in water ultrapure during 10 min by using a sonicator. In test tubes, the concentrations of SnO_2 (50,100 and 200 mg/l) were prepared in 10 ml of culture medium. For each tube we added 10 µl of paramecium, the culture was done at 28 ± 2° C. The total number of cells of paramecium was measured by the daily cell counting (0, 24, 48, 72 and 96 h), after fixation with formalin at 4%, under photonic microscope with enlargement 10 using grooved blade. The count was repeated at least three times for each sample.

Determination of Percentage of Response

Percentage of response is a reliable parameter to evaluate the xenobiotic effect via the inhibition of the cell growth of protists. Positive values of response percentage indicate an inhibition of growth, while negative values indicate a stimulation of growth (Bradford, 1976).

Percentage of response is calculated by the following formula:

$$Response(\%) = (CN - EN)/CN \times 100$$

Where **CN:** is the number of control cells (cell ml^{-1}) and **EN** is the number of treated cells (cell ml^{-1}).

Determination of Glutathione S-Transferase Activity (GST)

The measurement of Glutathione Stransferase (GST) is determined by the method of (Habig *et al.*, 1974). It is based on the reaction of conjugation between the GST and a substrate, the CDNB (1-chloro 2, 4 dinitrobenzene) and in the presence of a cofactor the glutathione (GSH). The absorbance was measured spectrophotometrcally at 340 nm.

Determination of Catalase Activity (CAT)

The measurement of Catalase activity (CAT) is determined using the method of (Regoli *et al.*, 1995). Whose principle is based on the change in consecutive optical density dismutation of hydrogen peroxide (H_2O_2). The (CAT) activity was determined spectrophotometrically at 240 nm

RESULTS AND DISCUSSION:

Structural Properties by XRD

The XRD pattern of the product is shown in figure 1.

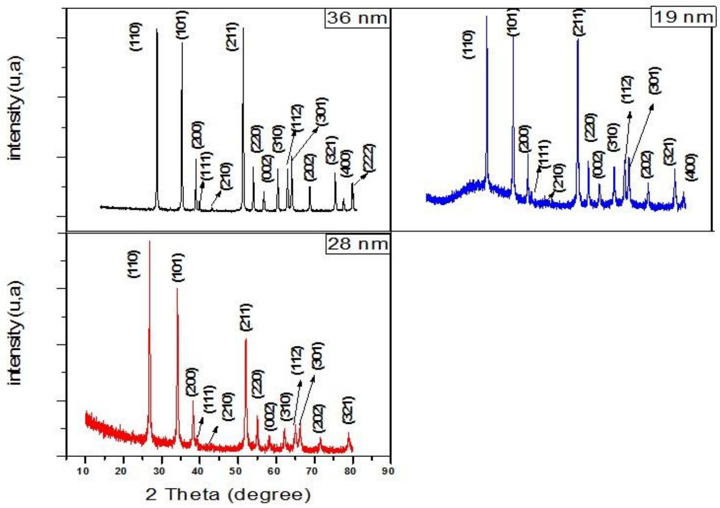

Fig 1 .The XRD patterns of the SnO_2 nanoparticles prepared by ball milling

The peaks pointed out at 2θ values of can be associated with (110), (101), (200), (111), (210), (211), (220), (002), (310), (112), (301), (202), (321), (400) and (222), respectively, correspond to tetragonal SnO_2 rutile structure crystallite (space group, P42/mnm), which are in good agreement with the literature values (JCPDS card no. 41–1445). The average size of the SnO_2 particles can be estimated by the Scherrer equation (Ganesh et al, 2012). **D= kλ /β cos θ**, where **D** is the crystallite size, **K= 0.9**, **λ** is the X-ray wavelength, **β** is the full width at half maximum of the diffraction peak, and **θ** is the Bragg diffraction angle of the diffraction peaks. The average size for the SnO_2 nanoparticles that were prepared by ball milling is shown in table. 1

Table 1 .
Average grain size obtained by different milling times (0, 1 and 3 hours)

Milling time (h)	0	1	3
Average grain size (nm)	36	28	19

FTIR Analysis

The FTIR spectrum of SnO_2 nanoparticles is shown in Fig. 2

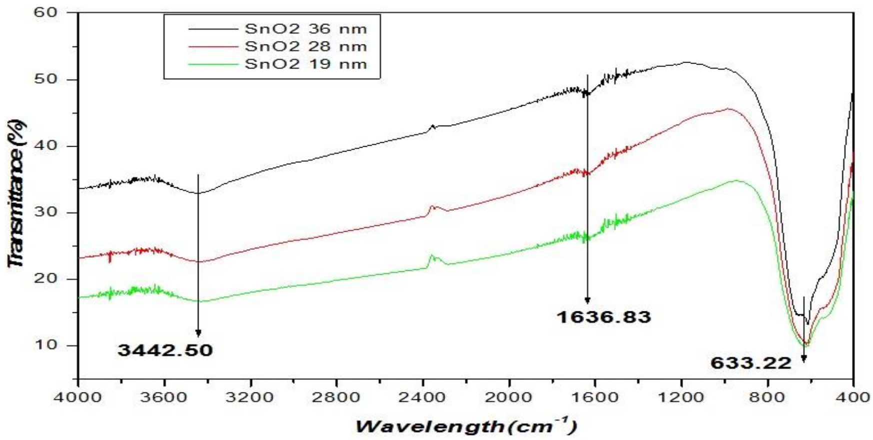

Fig 2. FTIR spectra of SnO_2 nanoparticles prepared by ball milling

From this spectrum, the peaks around 1628 and 3421 cm^{-1}corrospond to the binding vibrations of absorbed molecular water and the stretching vibration of –OH groups (Bagal *et al.*, 2012). A band which appeared in the range of 400 and 700 cm^{-1}, specifically, at 633.22 cm^{-1} is assigned to anti-symmetric Sn-O-Sn and anti-symmetric Sn-O stretching mode of the surface bridging oxide formed by condensation of adjacent surface hydroxyl group's vibration(Deosarkar *et al.*,2013). The absorption peak within 3300–3475 cm^{-1} (3442.50 cm^{-1}) is assigned to O–H stretching vibration of absorbed H_2O at the surface of the tin oxide (Mazloom *et al.*,2013).

Kinetics Growth

Figure.3 (a-b-c) shows changes in the number of *Paramecium tetraurelia* in function of time, treated with increasing concentrations of SnO_2 (50, 100,200 mg / l) with different grain sizes (36, 28, 19 nm) for each concentration.

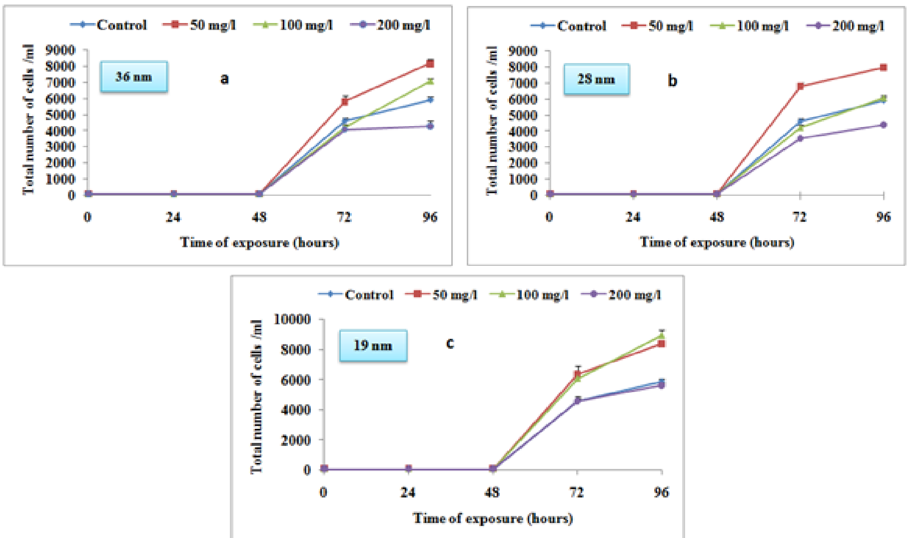

Fig 3(a- b- c). Effects of increasing concentrations of SnO_2 nanoparticles on the growth of *Paramecium tetraurelia* with different grain sizes (a 36 nm, b 28 nm , c 19 nm)

Response Percentage

Figure.4 (a-b-c) shows the percentage response *Paramecium tetraurelia* treated with increasing concentrations of SnO_2 (50, 100, 200 mg / l) with different grain sizes (36, 28, 19 nm), for each concentration. The results obtained concerning the response percentage confirm those of kinetics growth.

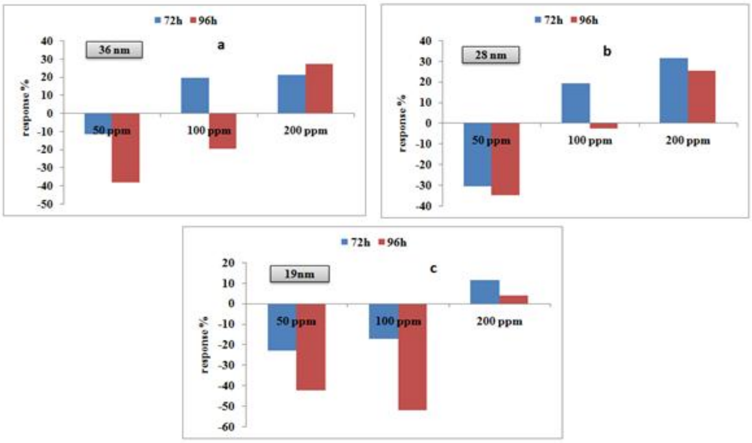

Fig 4 (a- b-c). Effects of increasing concentrations of SnO_2 nanoparticles on the response percentage with different grain sizes (a 36 nm, b 28 nm , c 19 nm)

Glutathione S-transferase (GST) Activity

Figure.5 (a-b-c) shows the changes in GST activity in *Paramecium tetraurelia* treated with increasing concentrations of SnO_2 (50, 100,200 mg / l) with different grain sizes (36, 28, 19 nm) for each concentration.

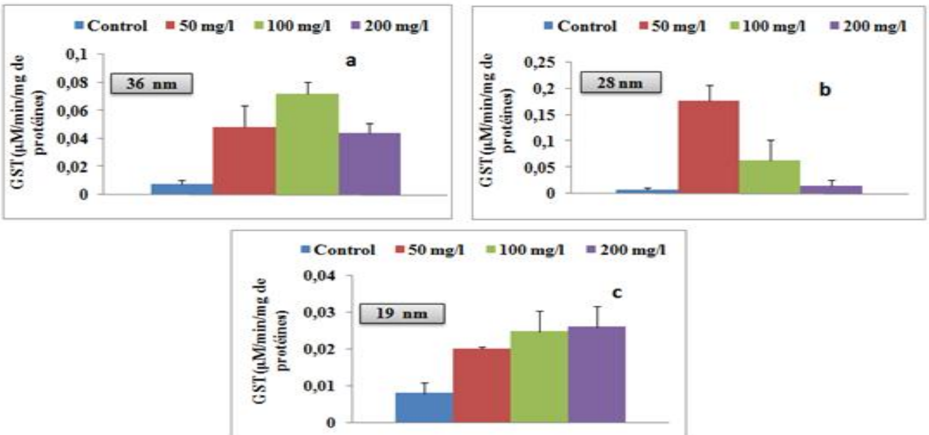

Fig 5(a-b-c). Variations of GST content in *Paramecium tetraurelia* exposed to increasing concentrations of SnO_2 nanoparticles with different grain sizes (a 36 nm, b 28 nm, c 19 nm)

Catalase Activity

Fig. 6 (a-b-c) shows the changes in Catalase activity in *Paramecium tetraurelia* treated with increasing concentrations of SnO_2 nanoparticles (50, 100, 200 mg / l) with different grain sizes (36, 28, 19 nm) for each concentration.

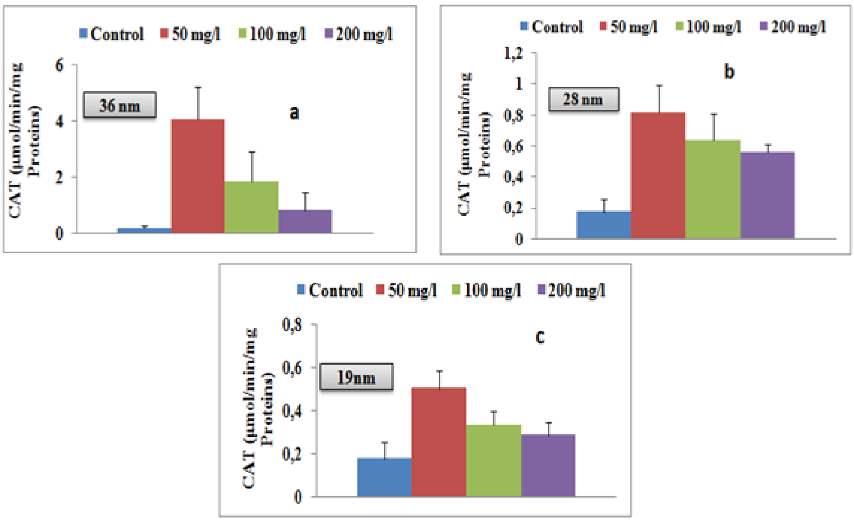

Fig 6(a-b-c). Variations of CAT activity in *Paramecium tetraurelia* exposed to increasing concentrations of SnO_2 nanoparticles with different grain sizes (a 36 nm, b 28 nm, c 19 nm)

Toxicity Discussion

Environmental pollution bioindicators are sensitive to physicochemical changes in their environment, such as hydrocarbons (Ismert *et al.*, 2002), trace metals (Gomot,1997), or pesticides (Coeurdassier *et al*.,2002; Vidal,2001 ; Rouabhi,2005), subjected to exogenous stress, micro-organisms have the ability to develop a battery capable of responses that trigger detoxification process, against xenobiotics to fight, and / or acclimatize against the chemical stress (Lagadic *et al.*,1997; Perez-Raman *et al.*,2001). Thus, protists are eukaryotic cells, ubiquitous in the aquatic and terrestrial environment, characterized by a short life cycle, rapid multiplication (Beal *et al.*, 1993) and any changes to their environment could affect their behavior, which justified the use of *Paramecium tetraurelia* as an alternative cell model for toxicology and risk assessment for health (**S**auvant *et al*., 1999). In this paper, we studied the toxicity of NPs SnO_2 of which toxic potential has been the main subject of our research. For that we were interested to investigate the effect of these NPs SnO_2 with different grain sizes (36, 28, 19 nm) on the growth kinetics of *Paramecium*, a parameter that actually reflects the toxicity of a xenobiotic (Perez-Rama *et al*., 2001; **S**auvant *et al*., 1999). The obtained results show that the exposure to low concentrations of NPs SnO_2 induces the stimulation growth of *Paramecium* for two concentrations (50 and 100 mg / l) and this is true for all grain sized (36, 28, 19 nm). We have also found that this stimulation was greater for the concentration 50 mg / l as that induced by a concentration of 100 mg / l for both particle sizes 36 and 28nm.

In contrary, high concentration of (200 mg / l) has obvious inhibition for two sizes of NPs grains (36, 28nm). On the grain size of 19 nm we have demonstrated a stimulation of *Paramecium* growth for both concentrations (50 and 100 mg / l); this stimulation is higher for the concentration 100 mg / l when induced by a concentration of 50 mg/l. As for the high concentration (200 mg / l), there was a very low inhibition.

The stimulation of cell growth for the two lower concentrations may be explained by the phenomenon of "hormesis", which can be defined: A dose-response relationship that is characterized by low-dose stimulation and a high-dose inhibition (Eaton *et al.*, 2001).

The work of (Shin *et al*., 2007), on the cytotoxicity of Ag NPs on peripheral blood mononuclear cells (peripheral blood mononuclear cells) has showed that low concentration of cell proliferation is stimulated and high concentration is inhibited (Shin *et al*., 2007), where (Jiao *et al.*, 2014), who observed that low concentrations of Ag NPs provoke a stimulation of cell proliferation of HepG2 cells.

The percentage of response is a parameter by which we can confirm the growth evolution curves of *Paramecium* treated by different concentrations of NPs SnO_2 used.

The antioxidant defense system is present in all aerobic cells; it neutralizes the intermediate chemical reactions produced endogenously and / or metabolism of xenobiotics. The activity of the antioxidant system may be induced or inhibited under the effect of a chemical stress (Winston *et al.*, 1991). Among the enzymes involved in the detoxification systems, our interest has focused on the development of Catalase activity, which is a catalyst for the reduction of hydrogen peroxide to water and molecular oxygen .This enzyme plays a role in preventing peroxidation of biological molecules and it is sensitive to certain contaminants inductor of oxidative stress at the cell membrane as the organic contaminants, but also metals and metal NPs (Buffet, 2012). Thus, our results show that for all the concentrations of NPs SnO_2, Catalase activity tends to increase and this true for all the tested grain size (19, 28, 36 nm). This bio-scoring has been very sensitive overlooked NPs. As suggested by (Buffet *et al*., 2011), who observed an induction of CAT activity in ***"****Scrobicularia plana"* bivalves and Annelid H diversicolore exposed to NPs CuO. On the other side (Pan *et al*.,2012) have demonstrated induction of CAT activity in *Scrobicularia plana* treated by Au NPs, or *"Mytilus galloprovincialis"* exposed to NPs SiO_2 and TiO_2 (Canesi *et al*.,2010) ,however for the highest concentration of NPs (100 and 200 mg / l), we found induction of CAT activity to be less important compared to treatment for concentration of 50 mg / l. (Gomes *et al.*,2011) explains this phenomenon by the ROS generated by NPs CuO, which induces an increase in CAT activity until reaching the limit of its antioxidant capacity then leading to its inactivation (Gomes *et al.*,2011).The other enzyme closely involved in the detoxification phenomenon is the Glutathione S-transferase. The GSTs are a large family of isoenzymes, part of the class II detoxification enzymes (Lukkari *et al*., 2004; Kim *et al*., 2009). They catalyze the conjugation of GSH with electrophilic cytotoxic compounds. The previous compounds and GSH conjugates are often less toxic, more water-soluble and can then be more easily expelled from the cell (Salinas *et al.*, 1999). GST plays a major role in the detoxification mechanism of species reactive oxygen species (ROS) and the regulation of redox equilibrium (Konings *et al.*, 1985; Siritantikorn *et al.*, 2007).

In this work, we have demonstrated the induction of GST activity for the three concentrations and for all grain sizes compared to the control. Our results confirm those of (Buffet *et al*., 2011), that showed induction of GST activity in *Scrobicularia plana* diversicolores and H exposed to CuO NPs and those of (Pan *et al*., 2012). S .plana exposed to NPs Au. This biomarker was also stimulated in *Mytilus galloprovincialis* exposed to SiO_2 NPs (Canesi *et al.*,2010) and in *Daphnia magna* exposed to NPs TiO_2 (Kim *et al*.,2009). We also found a lower GST activity for the concentration 200 mg / l for the grain size 36 nm while for the grain size of 28 nm depletion occurred in concentrations 100 and 200 mg / l, this can be explained by the fact that the GST has reached the limit of its antioxidant capacity then leading to inactivation as if was the case for Catalase activity. For the grain size of 19 nm we found an induction of GST activity for NPs treated with SnO_2.

CONCLUSION:

From the interpretation of obtained results in the present study, we can conclude that the low concentrations of SnO_2 nanoparticles (low cytotoxic concentration) had a beneficial effect on the growth of paramecium (activation of cell proliferation), as suggested by (Jiao *et al.*,2014) where two representative AGNPS with different particle sizes did not induced p38 MAPK pathway cytotoxic doses led to activation and promotion of the proliferation of HepG2 cell (Jiao *et al.*,2014), unlike the strong concentration where has been a decrease in cells growth. This can be explained by the phenomen on of hormesis, who is a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces year adaptive beneficial effect on the cell or organism (Calabrese, 2007; Mattson, 2008). Although the phenomenon of hormesis is considered a beneficial adaptive response, our results showed that despite the observed cell proliferation, this cannot prevent the paramecium of antioxidant system when activated and that at even low concentrations. For this and especially because of the complexity of potential interaction between NPs in general and NPs SnO_2 in our work and in living organism particulary, the potential long-term effect of its NPs at low doses on humans should be evaluated to establish standards for a safe and efficient use.

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