Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

The Impact of Individual and Combined Exposure to Aluminum and Chromium Particles on Oxidative Stress and Cellular Behavior in the Freshwater Ciliate Paramecium SP

Meriem Laouabdia-Sellami [1*], Khaled Belhamzaoui [1], Houria Berrebbah [1] and Mohamed Reda Djebar [2]
[1]Laboratory of Cellular Toxicology, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, Algeria.
[2]Environmental Research Center (ERC), Alzone 23000, Annaba, Algeria.

(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS
Mixture
Metals
Paramecium sp
Stress
Behavior.

ABSTRACT:

The cytotoxicity of metal mixtures has increasingly attracted the attention of scientists. In fact, living organisms are frequently exposed to complex mixtures of pollutants that arise especially as a result of industrialization. Combinations of these pollutants may have more harmful effects than the individual pollutants alone. Therefore, in order to accurately evaluate environmental studies, it is important to understand not only the toxicity of individual heavy metals, but also their interactions. In this context, the aim of this study is to evaluate the effect of four increasing concentrations (5µM, 10µM, 50µM and 100µM) of Aluminum and Chromium salts (separate and mixed) on a water pollution bio-indicator: the freshwater ciliate protist *Paramecium sp*. Our results confirm the generation of reactive oxygen species (ROS) by inducing oxidative stress after metal treatment. This is evidenced by stimulation of catalase and GST activities, as well as the increase in GSH and MDA levels. Furthermore, cell behavior analysis revealed sensitivity to these metals, especially their mixtures. This effect is illustrated by changes in its trajectory and interruptions in its speed of movement.

1. Introduction

Heavy metals can accumulate in the body, particularly in the kidneys. In fact, each type of metal has specific effects on human health. Nevertheless, the symptoms of toxicity appear in the event of absorption or contact with high concentrations of metals, or lower concentrations over the long term [1]. In fact, these molecules can transform and become potentially dangerous for human and environmental health by causing disruptive effects on various vital functions [2]. Various studies have been based on the analysis of waters contaminated by heavy metals, indicating a reduction in productivity and biodiversity, accompanied by qualitative and quantitative changes in populations above certain contaminant concentrations [3]. Aluminum is a light metal that occurs naturally in air, water and soil. Extracting and processing this metal raises its level in the environment [4]. As a result, it is also present in a number of products used frequently and daily, notably in cans, vaccines, cosmetics, toys, crockery, etc. However, its potential toxicity to human and environmental health is of growing concern to the scientific community. Previous research in the environmental toxicology field has revealed that Aluminium can pose a major threat to humans, animals and plants, causing numerous diseases [5]. In addition, many factors, including water pH and organic matter content, greatly influence aluminum toxicity. This is because Al absorption is optimal when the pH is acidic, which explains its high toxicity in acidic environments. This explains its high toxicity in acidic environments [6]. Moreover, aluminum toxicity might results from the interaction between aluminum and the plasma membrane, apoplastic and symplastic targets [7]. In humans, Mg^{2+} and Fe^{3+} are replaced by Al^{3+}, resulting in numerous disturbances associated with intercellular communication, cell growth and secretory functions. As a result, the alterations manifested in neurons are similar to the degenerative lesions observed in Alzheimer's patients. Consequently, the main complications of aluminum toxicity

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

are neurotoxic effects such as neuronal atrophy in the locus ceruleus, substantia nigra and striatum. Research by [8] confirmed the neurotoxicity of aluminum in laboratory animals, reinforcing concerns about its harmful effects on the human nervous system [9]. The Aluminum's toxicity has never ceased to worry the scientific community. Indeed, it has long been known that it is a toxic agent for freshwater aquatic organisms, especially in high concentrations, thanks to its dissolution in water, which creates bioavailable compounds. In the aquatic environment, it acts on gill animals, notably fish and invertebrates, by inducing a loss of plasma and hemolymphic ions, thus causing osmoregulatory failure, while retaining its capacity to bio-accumulate. Whereas in fish, the monomeric and inorganic form of Al can reduce important enzymatic activities. Aluminium also appears to accumulate in freshwater invertebrates. Thus, organically complexed Al can act synergistically with any number of other pollutants [10].

In nature, Aluminum is detected alone or in combination with other pollutant molecules, notably other metals, which mix and bind together, inducing greater effects on living organisms. Among the metals that can interact with Al are Chromium, whose hexavalent form is known to be the most toxic. In fact, the frequency of its release into the environment continues to increase over the years [11]. Its high hydro-solubility explains its mobility within ecosystems, where contamination by hexavalent Cr, the most toxic form, could spread, affecting various trophic levels. In fact, this Cr compound can affect aquatic fauna at concentrations of varying magnitude, as in the case of growth inhibition in freshwater phytoplankton at 10 µg-L-1. However, Cr is known to be of low toxicity in salt water [12,13]. Both metals are present in aquatic environments [14], which led us to choose the paramecium Paramecium sp as an excellent bio-indicator of aquatic pollution. This ciliated freshwater protist is an important link in the food web. It has been the subject of a great deal of research in Toxicology and Eco-toxicology, due to its ability to provide information on the quality of the environment, and to highlight the presence of any chemical pressure or contamination [15]. Its classifications are present, abundant and easily identifiable. Cultivation is simple, quick and cheap and its sensitivity provides information about each product tested. In addition, its contractile cilia are identical to those of mammalian epithelial cells, ensuring its mobility [16]. Thus, mammalian ciliary beats are perfectly comparable to those of paramecia (proven by high-speed microcinematography studies) [17]. Thanks to its organization, function and structural complexity, these cells can be used to analyze various biological, toxicological and eco-toxicological responses [18–20].

2. Methods

2.1. Chemical treatment:

Aluminum sulfate (Al) and potassium chromate (Cr) represents the main chemical molecules studied in our work. Besides, we were interested by the cytotoxicity induced by their mixture (Al/Cr). The used Aluminum and Chromium particles were respectively received from SAIDAL society and Chemistry department from The University of Annaba, Algeria. The concentrations of metals were selected after several tests: C1: 5µM, C2: 10µM, C3: 50 and C4: 100µM of each separated metal. For their combination, we choose these mixtures: C1Al/C1Cr: 5µM /5µM, C2Al/C2Cr: 10µM/ 10µM, C3Al/C3Cr : 50µM /50µM and C4Al/C4Cr : 100µM /100µM.

2.2. Cellular culture:

The specie of *Paramecium sp* were cultivated in the medium described by [21] composed primary of a mixture of vegetal infusions, including hay, wheat, potato, cucumber and peanuts, with the addition of a teaspoon of yeast. This mixture is brought to boil, then filtered and sterilized to be used as a culture medium of cells respecting the optimal growth conditions: pH: 6.5 (±2) and incubation temperature T°: 28°(±2). In the exponential phase, each 10µl sample of Paramecium is treated with the concentrations listed below and repeated at least three times.

2.3. Oxidative stress biomarkers:

In the beginning, the enzymatic activity of catalase (CAT) was determined following the method outlined by [22]. After cellular centrifugation at 15000 T/10min, a solution of 0.050ml of surnageant, 0.075ml of phosphate buffer and 0.2ml of H2O2 was disposed in a quartz cuvette. Then, the spectrophotometer lecture was realized at 240nm during 1 minute and the catalase activity was measured in nmol/min/mg of proteins. In addition, the measurement of Glutathione S-transferase (GST) followed the procedure described by [23] that involves the reaction of 200µl of enzymatic extract with 1,2ml of CDNB (1-chloro 2, 4-dinitrobenzène). The absorbance readings were taken every minute for 5 minutes at 340nm and the quatity of GST is expressed in µmoles/min/mg of proteins. Moreover, the levels of the glutathione (GSH) was determined according

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

to [24]. A cellular suspension was added to 1ml of EDTA (0.02M), then 0.8ml of each sample was mixed with 0.2ml of ASS solution (Sulfosalicylic Acid 0.25%) to protect the thiol groupment of Glutatione. After centrifugation, 0.5ml of the surnagent, 1ml of EDTA and 0.025ml of DTNB (5,5' dithio- 2- nitrobenzoic Acid) were placed in a plastic cuvette and measured the absorbance at 412nm after 5min of rest. In this case, the GSH level is expressed in µM/mg of proteins. Furthemore, the rate of Malondialdehyde (MDA) was quantified by the method established by [25]. This approach is based on the reaction between Thiobarbituric Acid (TBA) and MDA, resulting in the formation of a brown red product that is measured at 532nm. The Paramecia were homogenized in Tris HcL BUFFER (50 nM, pH 7,5) then centrifuged at 10000 T/10min. To initiate the process, 0.5ml of the surnageant is added to 2.5ml of TCA (Trichloroacetic Acid 20%). Following heating of the samples at 100°C, 1ml of TBA solution was introduced, and a second heating step was carried out. Subsequently, the addition of 1.5ml of Butanol became necessary. After the final centrifugation, the optical density (OD) readings were taken at 532nm, then the MDA concentration was determined and expressed in µmoles/mg of proteins. Therefore, all spectral measurements were performed using a JENWAY 3600 spectrophotometer.

2.4. Cellular behavior study :

Paramecium sp are excellent bio-indicators of environmental alterations, they have the capacity of changing their behavior when they are exposed to chemical contamination. Which represents the first early sign of cytotoxicity [26]. Indeed, in the presence of a xenobiotic in the environment, paramecia undergo perturbations in their movement trajectories and speed. These two parameters can be assessed using Kinovea software version 0.9.5, which enables the trajectory of movement to be traced precisely after analysis of the recorded videos. In this way, we can calculate the speed of movement by displaying the distance in pixels and the time in hundredths of a second. For this purpose, we recorded several videos of the cells treated with the highest concentrations (C4 of Aluminium (Al), C4 of Chromium (Cr) and C4Al/C4Cr) via microscopic observations (LEICA DM 1000) showing the movement of cells taken in the exponential phase on more or less straight trajectories. Thus, the video records were repeated at least three times.

2.5. Statistics:

The results obtained were analyzed with GraphPad Prism 9 software, using One way and Two ways ANOVA tests. Multiple comparison tests were also performed (dunnetts tests).Thus, the data obtained were presented as mean ± SEM standard deviation where significant differences were noted when $P \leq 0.05$ (significant : *), $P \leq 0.01$ (highly significant : **), $P \leq 0.001$ (highly significant : ***) and $P < 0.0001$ (very highly significant : ****).

3. Results

3.1. Evaluation of stress markers :

The obtained results presented in Tab.1 shows an important stimulation of the catalase activity in the cells treated with C3 of Al (0.00033 x104µmol/min/mg of proteins). A clear increase is noted in the cells treated with C3 and C4 of Cr (respectively 0.00046 x104 and 0.00037x104µmol/min/mg of proteins) compared to the control cells who do not exceed the value of 0.00013 x104µmol/min/mg of proteins. In fact, the ANOVA analysis indicates a very significant differences (p=0.0293). The Paramecium treatment with the metallic mixture (Al/Cr) induced an increase of the catalase activity especially in for those exposed to C2A/C2C and C1A/C1C with respectively the values of 0.0068 x104 and 0.00028 x104µmol/min/mg de proteins. However, the highly concentrations mixture (C4A/C4C) induced a decrease of catalase activity: 0.00007 x104µmol/min/mg of proteins. The results of Glutathione rates presented in Tab.1 shows a clear decrease in cells treated with Al where we can observe very high significant values for all the concentrations ($p<0.0001$) which C3 presented a value of 3 µmol/mg of proteins compared to the control cells 3.89 µmol/mg of proteins. In addition, the Cr treatment induced a very high significant decrease ($p<0.0001$) from a value of 1.274 µmol/mg of proteins (C1) to a value of 1.206 µmol/mg de proteins (C4). On the same way, the combination of Al/Cr induced a very high significant decrease of GSH quantity in cells treated from 0.204 µmol/mg of proteins in cells treated with C1A/C1C to 0.107 µmol/mg de proteins for those treated with C4A/C4C. The ANOVA analysis shows very high significant differences for either the Al, Cr or the mixture treatment. Thus, the monitoring of the GST activity shows an important increase in cells treated with Al. This induction is significant in the case of treatment with C2 ($p = 0, 0136$) with a value of 0,00079 µM/min/mg of proteins and a very high significant increase ($P<0.0001$) in the cells exposed to C4: 0, 0017 µM/min/mg of proteins compared to control cells (0,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

00017µM/min/mg of proteins). The results about Cr effect indicate a high significant increase (p= 0,0008) for C2 and a very high significant (p<0.0001) for C3 and C4 with respective values of 0,00028, 0,00030 and 0,00033 µM/min/mg. Also, the mixture treatment induced a very high significant increase in the *Paramecium sp* treated with C3A/C3C (p<0.0001) and very significant induction in those exposed to C4A/C4C (p= 0,0051) with a respective values of 0,00057 et de 0,00040 µM/min/mg of proteins. The ANOVA study indicated a high significant difference (p=0.0002). The quantification MDA level in cells treated with Al shows a dose-dependent increase with a value of 0.000176 µM/mg de proteins in the cells exposed to C1 and 0.000328 µM/mg de proteins (p= 0.0095) in the presence of the high concentration C4 : 0.000458 µM/mg de proteins (Tab.1). The ANOVA analysis shows a significant difference (p=0.0167). On the other hand, the Cr exposure also induced an increase of the MDA rate. In fact, this stimulation is important in the case of C2 and C3 treatment with respective values of 0.032 µM/mg of proteins and 0.035 µM/mg of proteins. The ANOVA study shows a significant difference (p=0.0142). The mixture of Al/Cr exposure induces a clear stimulation of MDA rate, which passes from a value of 0.0165 µM/mg de proteins in the cells treated with C1A/C1C to a value of 0.026 µM/mg de proteins in those exposed to C4A/C4C. In this case, the ANOVA analysis shows a very high significant differences (p<0.0001).

2.1. Effects on the cellular behavior:

The Fig.1 shows the different trajectories of control and treated cells with Al, Cr and their mixture. Kinovea's processing of the recorded videos reveals a clear shift in the movement trajectory of metal-exposed paramecia. First, we note that the control cells follow a linear trajectory in a helical fashion around a longitudinal axis (Fig.1-A). This direct movement is ensured by the waves created by the ciliary beats. However, in the case of exposure to the metals studied, we note a loss of linearity represented in Fig.2. Here we can see disordered trajectories following a ZigZag shape, a circular shape or an abrupt change of direction accompanied by backward swimming. In fact, all these alterations are manifested above all in cells treated with C4 Al salts (B), C4 Cr (C) particles and the combination of high concentrations (D). We also note a slight increase in displacement or the swimming speed in Al-treated cells (Fig.2), from a value of around 154.9mm/s for those treated with C2 to a value of around 224mm/s for those exposed to C4. However, we note a value of around 139.6mm/s for cells treated with C1, which represents a lower value than that observed in control cells (178mm/s). In addition, we also note an increase in the speed of movement of cells treated with potassium chromates (Fig.3). Indeed, this evolution is more pronounced in those exposed to the highest concentration (C4), reaching a value of around 188.4mm/s, compared to controls with a speed of around 178mm/s. Nevertheless, treatment of paramecia with the Al/Cr mixture induced a depletion of their displacement velocity (Fig.4). This decrease was significant for those treated with C1A/C1C, with a value of around 143.49mm/s (p= 0.0456), and highly significant for cells exposed to C2A/C2C, C3A/C3C and C4A/C4C, reaching values of around 130.6mm/s (p= 0.0061), 125.77mm/s (p= 0.0029) and 128.8mm/s (p= 0.0046) respectively. The ANOVA analysis of variance revealed a highly significant difference (p=0.0040).

Tab 1: Stress biomarkers (Catalase activity, Glutathione rate, Glutathione s-transferase activity and Malondialdehyde rate) in cells treated with Aluminum sulfates (Al), Potassium chromates (Cr) and their mixture (Al/Cr) compared to the control cells after the exponential phase.

		CAT activity (µmol/min/mg)	GSH rate (µmol/mg)	GST activity (µM/min/mg)	MDA rate (µmoles/mg)
	Control : 0µM	0,000136574 ±7,4248E-05	3,899 ± 0,0907	0,00017 ±0,000029	0,00458 ± 0,00282
Al	C1 :5µM	0,000141898 ±4,54682E-05	****2,037 ± 0,0301	0,00035 ±0,000117	0,00017 ±0,04504
	C2 :10µM	0,000123353 ±7,40234E-05	****2 ±0,05	*0,00079 ±0,000397	0,00050 ± 0,00862

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

	C3 : 50µM	0,000210117 ±0,000214668	****3 ±0,04	0,00058 ±0,000053	0,00107 ±0,048911
	C4 : 100µM	0,000143159 ±5,18945E-05	****2 ±4,8e-002	****0,0017 ±0,000190	**0,00032 ± 0,0090
Cr	C1 : 5µM	0,000194918 ±2,64068E-05	****1,274 ±0,049	0,00014 ±0,000008	0,0141 ±0,01091
	C2 : 10µM	0,000119264 ±6,62805E-05	****0,604 ±0,03	***0,0002 ±0,00002	0,032 ±0,01081
	C3 : 50µM	0,000467552 ±0,000271874	****0,770 ±0,035	****0,0003 ±0,00001	0,035 ±0,01049
	C4 : 100µM	0,000156723 ±0,000204566	****1,206 ±0,096	****0,0003 ±0,00003	0,023 ±0,01020
Al/Cr	5µM Al/5µM Cr	0,00028781 ±7,64998E-05	****0,2043 ±0,079	0,0002 ±0,00008	0,016 ±0,00050
	10µM Al/10µM Cr	0,000463833 ±0,000576584	****0,3026 ±0,158	0,0003 ±0,00010	0,02674 ±0,00851
	50µM Al/ 50µM Cr	0,000216532 ±0,000168975	****0,1110 ±0,022	****0,0005 ±0,00003	0,03726 ±0,00443
	100µM Al/ 100mM Cr	7,79872E-05 ±3,65338E-05	****0,1077 ±0,043	**0,0004 ±0,000036	0,0266 ±0,005300

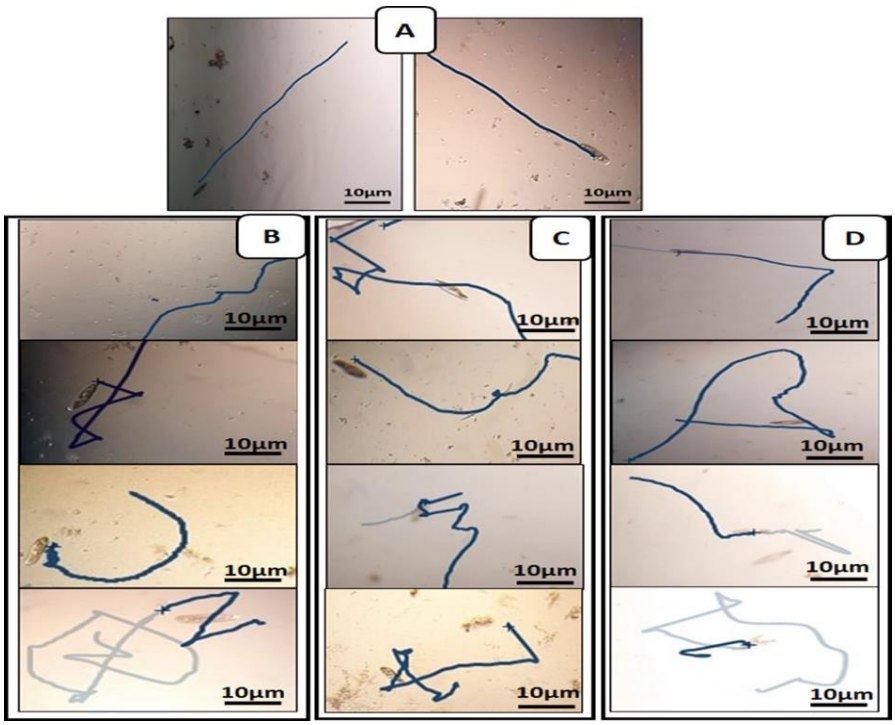

Fig 1: Different trajectories of the highest concentrations treated *Paramecium sp* filmed, analyzed and traced in blue line by Kinovea software (Grx10) after several repetitions. (A: Control, B: 100µM Al, C: 100µM Cr and D: 100µM Al/ 100µM Cr).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

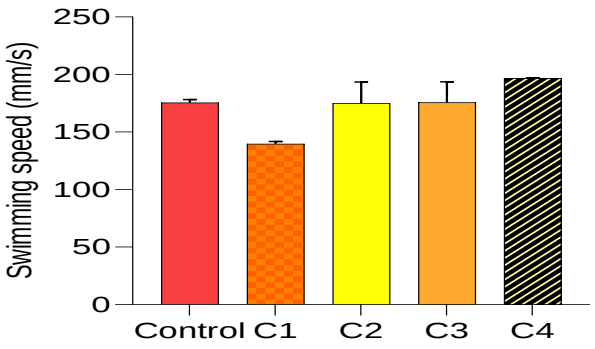

Fig 2: The Swimming speed (mm/s) of the treated cells with Aluminum sulfate during the exponential phase and using Kinovea software.

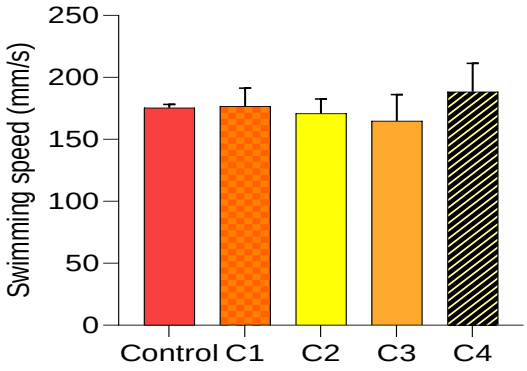

Fig 3: The Swimming speed (mm/s) of the treated cells with Aluminum sulfate during the exponential phase and using Kinovea software.

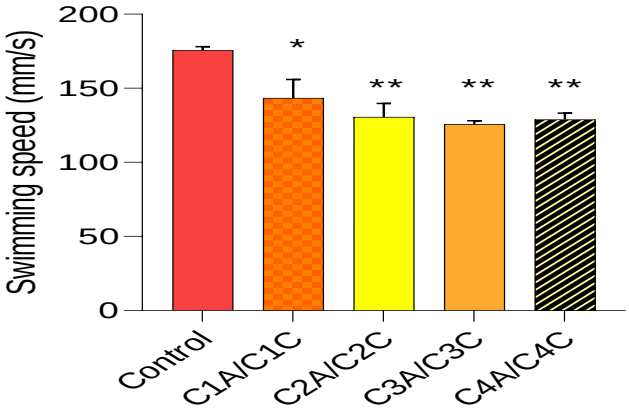

Fig 4: The Swimming speed (mm/s) of the treated cells with the mixture of Aluminum sulfate/ Potassium Chromate during the exponential phase and using Kinovea software. ANOVA analysis showed a very significant difference (p=0,0040).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

4. Discussion

In general, microorganisms are equipped with a xenobiotic defense system capable of overcoming stress conditions and maintaining cellular homeostasis [27, 28]. This system is activated when the effects of stress exceed homeostasis, calling on biomarkers, which designate all biochemical and physiological variations estimated in a living organism reflecting oxidative stress conditions [29].

In order to elucidate the involvement of stress caused by Aluminum, Chromium and their mixtures in the *Paramecium sp* and to study the physiological responses of this biological alternative model under unfavourable conditions, we were interested in estimating catalase activity, the first line of free radical defense. At the same time, we quantified GSH content, essential for ROS neutralization. We also monitored GST activity, a phase II biotransformation enzyme. Moreover, by assessing the level of MDA, the product of lipid peroxidation.

Firstly, we monitored catalase activity (CAT), a peroxisomal enzyme whose major role is to prevent hydrogen peroxide-induced peroxidation of biological molecules [30]. In fact, this enzyme is sensitive to contaminants that induce oxidative stress in cell membranes, such as PAHs, PCBs, certain pesticides and especially metals [31]. However, its response is considered irregular in vivo, as the results sometimes indicate stimulation and sometimes a decrease in this activity [32].

Secondly, we estimated the Glutathione (GSH) content of metal-treated yeasts and paramecia and their mixture. GSH plays a fundamental role in the intracellular defense process, neutralizing organic peroxide, helping to eliminate hydrocarbons by conjugation to the thiol group, and binding to heavy metal ions (Adam et al., 2005). Thus, any GSH deficiency exposes cells to a high risk of oxidative stress, due to its importance for the proper functioning of these detoxification enzymes: glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST)[33].

On the other hand, monitoring GST activity seemed essential. This enzyme belongs to a family of phase II biotransformation enzymes that detoxify exogenous substrates by conjugation with GSH to produce less toxic soluble compounds. In addition, it plays a key role in protecting against oxidative stress [34].

Assessing MDA levels has enabled us to study the phenomenon of lipid peroxidation, considered to be the most remarkable consequence of radical damage [35]. In the event of oxidative stress, the plasma membrane is the most important target for free radicals. The lipids that make it up oxidize and degrade, producing the end product Malondialdehyde, an early biomarker of lipid peroxidation [36]. The end result is a loss of membrane permeability and potential, and inactivation of membrane receptors and enzymes, leading to DNA damage and cell death [37].

The results reported in our work highlight a significant increase in catalase activity, GSH levels, GST activity and MDA content in the two cell models studied and following exposure to the two metals and their mixture (AL/Cr).

The reported induction of CAT activity, GST and MDA content is fully in line with the work of many scientists including [38–41] who assessed the toxicity of Medicines, Nanoparticles, Pesticides, heavy metals on different biologic models such us : *S.cereviciae*, *Paramecium sp*, *Daphnia magna* and some Coelomates.

The same applies to the work of [42], who highlighted CAT activity in Daphnia magna in the presence of cadmium and copper, underlining their potential to generate free radicals. With regard to the elevation of MDA levels, our results also confirm those of [43] who noted a significant induction of MDA after 48 h of exposure in *T. pisana* snails contaminated with Cadmium and Zinc. In addition, studies by [44] based on earthworm exposure to various heavy metals showed the same results.

However, the increase in GSH found in our results corroborates the findings of [45], who noted an increase in GSH in Fe2O3-treated *Helix aspersa*. In fact, the interaction of toxic metals with GSH metabolism is an essential part of the response of many metals [46]. When GSH is depleted by any metal, GSH synthesis systems begin to produce more GSH from cysteine via the g-glutamyl cycle [47]. In contrast, various studies have highlighted a depletion of GSH content under stress. [43] suggest that a decrease in GSH levels is due to its use in the conjugation of electrophilic compounds by GST during exposure to contaminants, alongside its use in the formation of specific thiol complexes with metal ions. In our case, the increase in Glutathione content is explained by the presence of a certain affinity between GSH and

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

metals, leading to the formation of GSH oxidation complexes. Moreover, GSH's contribution to the synthesis of metallothionins, responsible for metal chelation, also explains its induction within the cell [48]. The identification of oxidative stress biomarkers reveals mitochondrial damage the seat of cellular respiration and antioxidant enzyme synthesis. Our results are in line with those of [49] and [50], who claim that frequent exposure to Al is often accompanied by mitochondrial dysfunction. Or those of [51] who worked on various laboratory animals and deduced that Al treatment strongly induces the phenomenon of oxidative stress.

The achievement of RedOx equilibrium highlighted in our work is in line with the results of [52], who revealed the generation of ROS by Aluminum in *S.cereviciae*, inducing Superoxide dismutase (SOD) and Catalase (CAT) activity in parallel with a disruption of GSH and TBARS levels. Furthermore, the effects recorded following exposure to potassium chromate are in line with the findings of [53], who confirms the ROS-generating power of chromium in three types of cereal (durum wheat, common wheat and barley), leading to various biochemical, enzymatic, physiological and morphological disturbances in plants.

Paramecia are protists with the ability to change their behavior, including trajectories and speeds, in the presence of an exogenous agent. This makes them an ideal model for toxicology and eco-toxicology research. In fact, any perturbation in their behavior can highlight the different fluctuations induced by any contaminant present in aquatic environments. In fact, [54] states that paramecium trajectories are generally helical around a straight axis. However, in the presence of several stimuli, these trajectories can change. When the cell encounters no obstacle, it moves straight ahead; if an object lies on its axis, it changes direction. What's more, when the stimulus comes from all sides, the paramecium follows a random direction, with abrupt changes in trajectory.

With regard to movement speed, [55] reports that [56] explains the presence of three different swims and speeds. In fact, following a weak stimulus, cell swimming is normal with a stable movement speed. When the stimulus is stronger, the ciliary beats are faster, inducing an acceleration in the movement of the paramecia. Finally, when the stimulus is strong and brutal, the cell develops a defense system that does not depend on ciliary beats but on the sudden release of trichocysts towards the object of the stimulus, triggering a leap that enables the paramecium to escape the aggression.

Our results show a disruption of the trajectory and a slowdown in the speed of movement of cells exposed to the two metals and their combination. These results are in line with those of [57], who reported a disruption of swimming motion in paramecia in the presence of high concentrations of lead. This is fully in line with the results reported by [58], who showed a sudden reduction in motility followed by immediate cell lysis in *P. caudatum* exposed to increasing concentrations of graphene oxide (GO) nanoparticles. Also, those of [59], this author recorded a decrease in movement speed, backward swimming, pivoting, circling and forward movement in a new direction following exposure of *Paramecium bursaria* to Nickel Chloride. Thus, our results corroborate those of [60] who noted a disturbance in the speed of movement of paramecia after bringing them into contact with the mixture of nanoparticles and pesticides. In a similar manner, [61] observed that paramecia exposed to clay nanoparticles experienced a disruption in their displacement speed. Furthermore, the studies of [62] showed the impact of combined metals (Cobalt and Nickel) on Paramecium, inducing significant toxicity and metal accumulation within the cells.

Based on our data, which have been compared with the literature, we suggest that Al, Cr and especially their mixture act by altering Paramecium mitochondria by the stress generation, and its cytoplasmic membrane polarization by disturbing its movement and speed, which mainly results from the disruption of calcium flux flow within Ca2+ and K+ channels. Since that, the mitochondria are known to be involved in cell signalling [63], one consequence of mitochondrial alteration may actually be the disruption of membrane polarization.

5. Conclusion:

In conclusion, it is crucial to highlight the impact of the two metals (Al and Cr) and especially their mixture (Al/Cr) on the state of stress in Paramecium sp. This effect is demonstrated in our results, which indicate an evolution of catalase activity, which represents the first line of antioxidant defense, and an induction of GSH levels, an indispensable non-enzymatic biomarker. Accompanied by a stimulation of GST activity, which testifies to the fact that these molecules are bio-

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

transformed within the cell, and a remarkable increase in MDA content, a bio-indicator of lipid peroxidation that translates the chemical damage leading to cell death. In addition, the study of paramecium behavior in the presence of metals has enabled us to deduce their effects on cell structure and signalling. Indeed, the disruption of trajectories and speed of movement is strongly linked to the movement of intra- and extra-cellular calcium particles.

References:

1. Squinazi F., 2020. Synthèse : Les effets du plomb sur la santé, EnvironnementRisques& Santé, 5 (1) : 26.
2. Kalia K., Flora S. J., 2005.Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning, Journal of occupational health, 47(1):1-21.
3. Granum E., Raven J. A., Leegood R. C., 2005. How do marine diatoms can fix 10billiontonnes of inorganic carbon per year? , Journal of Botany, (83):898-908.
4. Agency for Toxic Substances and Disease Registry (ATSDR)., 2008. Toxicological profile for Aluminum. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
5. Barabasz W., Albinska D., Jaśkowska M., Lipiec J.,2002. Ecotoxicology of Aluminium. Polish Journal of Environmental Studies, 11.
6. Boudjellal, A., El-Hadef El-Okki, M., Al Mualad, W.N.A., Allam, O., Bader, R., Bassi, N., Benguedouar K., Benhamiche H., Benhamimid H., Boudaoud C., Boussekine R., Bouziane M., Kheroufi, A., Ksier, D., Makhlouf, F.Z., Mansouri, S., Mekaoussi, I., Meradji, M., Messioud, Y., Moali, M., Mouzai, A., Sahli, K., Sayah, T.M., Agli, A. 2022. Impact de l'aluminium sur la santé humaine. Algerian Journal of Nutrition and Food Sciences, 2(1), 1–16.
7. Kochian L.V., Piñeros M.A., Hoekenga O.A., 2005.The Physiology, Genetics and Molecular Biology of Plant Aluminum Resistance and Toxicity. Plant Soil 274, 175–195.
8. Yokel RA., 2002. Aluminum chelation principles and recent advances. Coord. Chem. Rev, 228 : pp. 97-113.
9. Hoet P., 2015. Chrome et composés. EMC - Pathologie professionnelle et de l'environnement ;10(3):1 11.
10. Rosseland B.O, Eldhuset T.D, Staurnes M.,1990. Environmental effects of aluminium. Environ Geochem Health, 12(1-2):17-27.
11. Xia S., Song Z., Jeyakumar P., 2020. Characteristics and applications of biochar for remediating Cr(VI)-contaminated soils and wastewater. Environ Geochem Health 42, 1543–1567.
12. Chiffoleau J.C., 2001. La contamination métallique, IFREMER, Région Haute Normandie. (Programme scientifique Seine Aval), 8, 39.
13. Ben Khelil M., Ben Said O., Nefzi R., Ben Abdelmalek J., Aïss P., 2012. Étude expérimentale de l'évolution en microcosme du chrome (VI) dans l'eau de mer et de ses effets sur la macrofaune et les bactéries indigènes de la Lagune de Bizerte (Tunisie). Revue des sciences de l'eau / Journal of Water Science, 25(3), 221–234.
14. Food and Drug Administration, 2002.Aluminum in large and small volume parenterals used in total parenteral nutrition; amendment; delay of effective ,67:70691–70692.
15. Shunmugam A.P., Subramanian G., Fernandez J. G., 2021. Measurements of the swimming speeds of motile microorganisms using object tracking and their correlation with water pollution and rheology levels. Sci Rep 11, 11821.
16. Gräf F., Stahr K., 1999. A Soil and Terrain Map of SW-Niger (NiSOTER). In: Atlas of Benin and Niger.
17. Berrebbah, H., 1990. Etude du contrôle de la mobilité flagellaire et ciliaire par le calcium. Effet du lindane, insecticide organochloré. Thèse de Doctorat de l"Université Pierre et Marie Curie, Paris VII, 144 p.
18. Meyer E., Beisson J., 2005. Épigénétique: la paramécie comme modèle d'étude. Medecine/Sciences, vol 21, n°3, 377-83.
19. Bouaricha H., 2013. Evaluation du stress oxydatif induit par le Proclaim : Essai comparatif sur deux modèles biologiques (Helix aspersa et Paramecium sp.). Thèse de doctorat, Université Badji Mokhtar, Annaba, Algérie, 55.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

20. Shaikh H.M.S., Pimpliskar M.,2022. Effect of heavy metals on paramecium found in different water bodies of Bhiwandi. IP Int J Med Microbiol Trop Dis, 8(2), 127-131.
21. Azzouz Z., Berrebbah H., Djebar MR., 2011. Optimization of Paramecium tetraurelia growth kinetics and its sensitivity to combined effects of azoxystrobin and cyproconazole. African Journal of Microbiology Research, Vol 5, 3243-3250.
22. Clairborne, A., 1985. Catalase activity. Handbook of methods for oxygen radical research CRC. Press Boca. Raton, Florida. 283-284.
23. Habig WH., Pabst MJ., Jakoby WB., 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. Journal of Biological chemistry, 249,7130-7139.
24. Wechbeker G., Cory., 1988. Ribonucleotidereductase activity and growth of glutathione depleted mouse leukemia L1210 cells in vitro. Cancer letters, 40, 257264.
25. Draper H.H., Hadley M., 1990. Malondialdehyde determination as index of lipid peroxidation. Meth. Enzymol. 186, P.241-431.doi: 10.1016/0076-6879(90)86135-i.
26. Burgeot T., Bustamante P., Fort J., Coutellec M.A., Azam D., Olivier G., Chaumot A., Armengaud J., Maltby L., Minier C., 2020. Etude de la qualité des milieux aquatiques : Caractérisation des impacts écotoxiques. ISTE éditions, 70 p.
27. Wassmann S., Wassmann K., Nickenig G., 2004. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension, 44(4), 381-386.
28. Mouneyrac C., Buffet P.E., Poirier L., Zalouk-Vergnoux A., Guibbolini M., Risso-de Faverney C., Perrein-Ettajni H. Fate and effects of metal-based nanoparticles in two marine invertebrates, the bivalve mollusc Scrobicularia plana and the annelid polychaete Hediste diversicolor. Environmental Science and Pollution Research, 21 (13), 7899, 2014.
29. Huggett R. J., Stegeman J. J., Page D. S., Parker K. R., Woodin B., Brown J. S., Bence A. E., 2004. Biomarkers in fish from Prince William Sound and the Gulf of Alaska: 1999–2000. Marine Environmental Research, 58, 305-325.
30. Greulich K., Hoque E., Pflugmacher S., 2002. Uptake, metabolism, and effects on detoxication enzymes of isoproturon in spawn and tadpoles of amphibians. Ecotoxicology and environmental safety, 52(3), 256-266.
31. Labrot F., Ribera D., Denis M. S., Narbonne J. F., 1996. In vitro and in vivo studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinesterase, catalase and glutathione peroxidase activities in three non-mammalian species. Biomarkers, 1(1), 21-28.
32. Di Giulio R. T., Habig C., Gallagher E. P., 1993. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. Aquatic toxicology, 26(1-2), 1-22.
33. Kizek R., Vacek J., Trnková L., Jelen F., 2004. Cyclic voltammetric study of the redox system of glutathione using the disulfide bond reductant tris (2-carboxyethyl) phosphine. Bioelectrochemistry, 63(1-2), 19-24.
34. Wu Q. S., Xia R. X., Zou Y. N., 2006. Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (Poncirus trifoliata) seedlings subjected to water stress. Journal of plant physiology, 163(11), 1101-1110.
35. Favier A., 2003. Le stress oxydant. L'actualité chimique, 108(10), 863-832.
36. Viarengo A., Lowe D., Bolognesi C., Fabbri E., Koehler A., 2007. The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146(3), 281-300.
37. Marnett LJ., 2000.Oxyradicals and DNA damage. Carcinogenesis 21:361–3701.doi: 10.1093/carcin/21.3.361.
38. Cherait A.Toxicity evaluation at the cellular and subcellular level of a compound from the dihydropyredine family on an experimental stress bioindicator model. Doctoral thesis, University of Annaba, Algeria,2015.
39. Khebbeb M. Evaluation of the toxicity induced by ZnO nanoparticles on two unicellular biological models Saccharomyces cereviciae and Paramecium

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 2305-2316 | ISSN:2251-6727

sp. Doctoral thesis, University of Annaba, Algeria, 2016.
40. Moumeni O., 2016. Etude de la toxicité d'un cyclohexanedione (herbicide) sur deux modèles biologiques d'eau douce : Paramecium tetraurelia et Daphnia magma. Thèse de doctorat, Université Badji Mokhtar, Annaba, Algérie, 171p.
41. Othmani H., 2018.Evaluation de la toxicité de quelques métaux lourds sur le comportement d'un modèle animal du groupe des Coelomates. Thèse de Doctorat, Université Badji Mokhtar, Annaba,Algérie.
42. Barata C., Varo I., Navarro J. C., Arun S., Porte C., 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 140(2), 175-186.
43. Radwan M. A., El-Gendy K. S., Gad A. F., 2010. Biomarkers of oxidative stress in the land snail, Theba pisana for assessing ecotoxicological effects of urban metal pollution. Chemosphere, 79(1), 40-46.
44. Yang X., Song Y., Kai J., Cao X., 2012. Enzymatic biomarkers of earthworms Eisenia fetida in response to individual and combined cadmium and pyrene. Ecotoxicology and environmental safety, 86, 162-167.
45. Besnaci S., Bensoltane S., Djekoun M. Oxidative stress and histopatological changes induced by the nno-Fe2O3 in Helix aspersa. Scientific Study & Research, 20 (2),119, 2019.
46. Hultberg B., Andersson A., Isaksson., 2001. Interaction of metals and thiols in cell damage and Glutathione distribution: potentiationofmercury toxicity by dithiothreitol. Toxicology, 156: 93-100.doi: 10.1016/s0300-483x(00)00331-0.
47. Quig D., 1998. Cysteine metabolism and metal toxicity, Altern Med Rev,3(4):262-70.
48. Regoli F., Principato G., 1995. Glutathione, glutathione-dependent and antioxidant enzymes in mussel, Mytilus galloprovincialis, exposed to, metals under field and laboratory conditions: implications for the use of biochemical biomarkers, Aquatic Toxicology, 31, 143-164.doi.org/10.1016/0166-445X(94)00064-W.
49. Wu Z., Du Y., Xue H., Wu Y., Zhou B., 2012. Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. Neurobiology of aging, 33(1), 199-e1.
50. Iglesias-González J., Sánchez-Iglesias S., Beiras-Iglesias A., Méndez-Álvarez E., Soto-Otero R., 2017. Effects of aluminium on rat brain mitochondria bioenergetics: an in vitro and in vivo study. Molecular neurobiology, 54, 563-570.
51. Kumar V., Gill K. D., 2009. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Archives of toxicology, 83, 965-978.
52. Chen R., Zhu Q., Fang Z., Huang Z., Sun J., Peng M., Shi P. Aluminium induces oxidative damage in Saccharomyces cerevicia. 2020.
53. Alayat A., 2015. Etude de l'Impact toxicologique de certains agents chimiques sur la qualité des céréales: cas du Blé et de l'Orge, Thèse de doctorat. Université Badji Mokhtar. Annaba, Algérie.
54. Machemer H., 2001. The swimming cell and its world: Structures and mechanisms of orientation in protists. Eur. J. Protistol. 37: 3-14.
55. Azzouz, Z., 2012. Etude des effets toxiques d'un fongicide (Amistar Xtra) et d'un herbicide (Glyphosate) sur la biologie et le comportement de Paramecium tetraurelia. Thèse de Doctorat, Université Badji mokhtar, Annaba, Algérie, 141p.
56. Hamel A., Fisch C., Combettes L., Dupuis-Williams P., Baroud C.N., 2011. Transitions between three swimming gaits in Paramecium escape. Proc. Natl. Acad. Sci. USA. 108(18): 7290-7295.
57. Bernal J., Ruvalcaba S., 1996. Pharmacological prevention of acute lead poisoning in Paramecium. Toxicology, 108(3), 165-173.
58. Armus H. L., Montgomery A. R., Jellison J. L., 2006. Discrimination learning in paramecia (P. caudatum). The Psychological Record, 56, 489-498.
59. Zagata P., Kopańska M., Greczek-Stachura M., Burnecki T., 2015. Acute toxicity of metals: Nickel and Zinc to Paramecium bursaria and its endosymbionts. Journal of Microbiology, Biotechnology and Food Sciences, 128-131.
60. Bouraiou C., 2022. Effets combinés de nanoparticules de CuO et du Chlorpyrifos (organophosphoré) sur certains modèles alternatifs :

Paramecium sp et Daphnia magna, Thèse de Doctorat, Université de Annaba, Algérie.

61. Tarkington L., Bryan M., Kolhatkar Tejas W., Markle Nathanael W., Elizabeth J., Raska A., Michael M., Cubacub., Supparesk Ritti kulsittichai., Chien-Hung Li., Yi-Ting Chen., Andrew Jamison, C., 2016. Magnetic Microorganisms: Using Chemically Functionalized Magnetic Nanoparticles To Observe and Control Paramecia. American Chemical Society and Division of Chemical Education, Inc. J. Chem. Educ. 2017, 94, 85−90.
62. Garza Amaya D. L., Thiel A., Möller, M., Gasparoni G., Pirritano M., Drews F., Simon M., 2023. Nickel and Cobalt Induced Toxicity in Paramecium: The Cellular View to Environmental Toxicants, 2-29.
63. Picard M., Shirihai O. S., 2022. Mitochondrial signal transduction. Cell metabolism, 34(11), 1620-1653.