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Change in the spatial organization of mussel mitochondria exposed to polystyrene nanoplastics

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ABSTRACT

Mitochondria are central for cellular energy metabolism, respiration and production of reactive oxygen species (ROS) making them easy targets for xenobiotics. Plastic nanoparticles (NPs) represent an emerging threat to exposed organisms and their effects were examined in mitochondria. The goal of this study was to examine changes in the fractal dimension of mitochondria caused by selected environmental contaminants such polystyrene nanoparticles (NPs). Mitochondria from *Elliptio complanata* mussels were isolated and exposed *in vitro* to selected environmental chemicals: nicotine (Nic), isoniazid (Iso), ibuprofen (Ibu), erbium (Er), lutetium (Lu), zinc oxide and polystyrene NPs for one h at 25 °C. The activity in NADH oxidase, the oscillatory behavior in NADH levels and the fractal dimension were determined during that time. The following compounds were able to accelerate the reaction rate of NADH oxidase, which are the main source of ROS in cells: Nic and Iso. Zinc oxide NP decreased NADH oxidase. Nic and Iso also reduced the fractal dimension (fD) of NADH changes, which was consistent with the nicotinic acetylcholine receptor pathway in blocking mitochondrial permeability transition pores. NADH levels oscillated during the exposure period and revealed a frequency dependent decay in amplitudes consistent with a fractal environment. Nic, Iso and polystyrene NPs were able to decrease the fD and the appearance of low amplitude frequencies. The fractal properties of NADH changes in time revealed that reduced dimensions leading to increased NADH oxidase activity by polystyrene NPs. This could form the basis of polystyrene NP-induced oxidative stress in organisms.

KEYWORDS: mitochondria, permeability transient pore, nicotine receptors, polystyrene nanoplastics.

1. INTRODUCTION

Mitochondria are the central plexus responsible to energy metabolism and cellular respiration in organisms. They are highly complex and dynamic structures that are fundamental in cellular physiology and metabolism. Mitochondria are responsible for glucose metabolism producing electrons for energy production (ATP production) and respiration ($O_2 \rightarrow CO_2$). NADH chemically stores electrons during the tricarboxylic acid cycle which, in turn, are transferred to the electron transport system (Complex 1) for the synthesis of ATP in cells. Disturbances in this delicate balance between NADH production and NADH oxidation for electron transporting activity are involved in many pathophysiological conditions, degenerative diseases and ageing [1]. Both mitochondrial and bacterial complex I of the electron transport system catalyzes the oxidation of NADH in the presence of oxygen producing highly toxic reactive oxygen species (ROS) [2]. The production of ROS represents in the order of 0.2-0.3% of the oxygen consumed for metabolism and respiration. ROS are eliminated by a suite of non-enzymatic and enzymatic antioxidant pathways such as superoxide dismutase, catalase, ascorbate and reduced glutathione [3].

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The capacity of toxic chemicals to disrupt this delicate balance and initiate toxicity leading to mitochondria dysfunction and apoptosis is well established [4]. Mitochondria possess nicotinic acetylcholine receptors not only in excitable cells but in non-excitable cells as well [5, 6]. In non-electrical cells these receptors are involved in the maintenance of permeability transient pores and voltage dependent anion channels involved in the maintenance of Ca^{+2}. If uncontrolled, Ca^{2+} accumulates in mitochondria leading to swelling and release of cytochrome c, an initiator of apoptosis. Recent studies revealed that nicotinic acetylcholine receptors could regulate the formation of mitochondrial permeability transition pore involved in the release of apoptotic mediators such as ROS and cytochrome c [7]. These receptors do not need ion gradients to activate voltage dependent anion channels. Mitochondria are therefore susceptible targets to nicotinic compounds [5], some of which could be released in the environment by treated municipal wastewaters from tobacco use [8]. Mitochondria are excellent surrogates in ecotoxicological studies because they are highly sensitive to both chemical and physical stresses [9, 10]. Mitochondria are complex multifonctionnal units involving highly crowded environments with fractal properties [11], i.e. producing patterns at different scales. The cell is considered a fractal multioscillator i.e., oscillations are found at many levels of biological organization. Indeed, NADH levels and oxygen consumption can oscillate in mitochondria [12]. This process results from the temporal partitioning of NADH production and NADH oxidation (ROS levels) during respiration and glucose metabolism. It involves other intermediates such as citrate, succinate, K^+ and Ca^{2+}. Polystyrene NPs are thought to be chemically inactive but the introduction of non-polar surfaces (styrene polymers) and crowding effects of NPs within cells or mitochondria could lead to alteration in the spatial organization of mitochondria, which is of fractal nature [11].

These oscillations result from the dynamic control between NADH formation during the aerobic glycolysis steps (tricarboxylic acid cycle) and NADH oxidation for electron transport activity to support ATP synthesis and the control of ROS production. In theory, compounds that cause oxidative stress and reduce the capacity to remove the normal production of ROS during respiration could disrupt the oscillatory behaviour of mitochondria [13]. The dampening in sinusoidal oscillations is a consequence of the loss of internal balance between NADH production and oxidation in mitochondria and from desynchronization of mitochondria.

The purpose of this study was to examine the fractal behavior of NADH oscillations in mitochondria exposed to relevant environmental contaminants in the attempt to identify novel biomarkers of effects for polystyrene NPs. Mitochondria were exposed to environmentally relevant urban contaminants and those known to introduce volume changes in mitochondria (nicotine). These responses were then compared with the observed biophysical effects of polystyrene NPs.

2. MATERIALS AND METHODS

2.1. Freshwater mussel collection and handling

Adult *Elliptio complanata* (6 ± 2 cm long) were collected at a remote (pristine) lake in the Laurentians during the summer (June) of 2017. They were immediately transported to the laboratory in humidified coolers. The mussels were placed in a 60 L aquarium with dechlorinated, UV-treated tap water (City of Montreal) for 4 weeks at 15 °C under constant aeration. The mussels were fed daily with concentrates of commercial phytoplankton suspensions (Phytoplex, Kent Marine, WI) and in-house preparations of *Pseudokirchneriella subcapitata* (2 L of 50-200 million cells/mL).

Mussels (n = 8) were randomly collected and their soft tissues were removed, weighed and homogenized at a 1:5 (w/v) ratio in ice-cold 10 mM Hepes-NaOH (pH 7.4) containing 200 mM sucrose, 1 mM EDTA and 1 µg/mL apoprotinin. Homogenisation was achieved using a Teflon pestle tissue grinder (5 passes at 4 °C). The homogenates were then centrifuged at 1500×g for 15 min at 2 °C and the supernatant was centrifuged again but at 10 000×g for 30 min at 2 °C. The mitochondria pellet was resuspended in 200 mM sucrose containing 10 mM Hepes-NaOH, pH 7.4, 1 mM KH_2PO_4 and 1 mM $NaHCO_3$ and stored at −85 °C until analysis. Total protein levels were determined according to the method of Bradford [14] using standard solutions of serum bovine albumin for calibration. Proteins

were diluted in 10 mM NaOH and appropriate blanks and standards were used.

2.2. NADH oxidation and oscillations

The initial NADH oxidation rates and long-term oscillations were determined as previously described [13]. Briefly, mitochondria preparations were diluted to 0.1 mg/mL in 200 mM sucrose containing 10 mM Hepes-NaOH, pH 7.4, 0.1 mM $MgCl_2$, and 1 mM KCl. After 5 min, 0.1 mM NADH was added to the suspension, and fluorescence measurements at 360 nm excitation and 460 nm emission were taken every 2 min for 10 min at 30 °C. The assay was performed in 200 µL volume in dark microplates with a fluorescent microplate reader (Synergy-4, Biotek Instruments, USA). The oxidation rate was reported as a decrease in NADH fluorescence x (min^{-1} mg^{-1}) of protein within the first minutes (initial conditions). Oscillations between reduced NADH and oxidized NAD^+ in mitochondria were also measured in dark 96-well microplates as follows. Mitochondria were diluted to 0.1 mg/mL total protein with 200 mM mannitol, 25 mM sucrose, 5 mM KH_2PO_4, 1 mM $NaHCO_3$, 2 mM $MgCl_2$, 1 mM K-ADP, 2 mM pyruvate and 5 mM Hepes-NaOH (pH 7.4). Fluorescence readings were taken every 30 s for 45 min in sweep mode (excitation using 10 msec flash and emission readings with no delay) using the Synergy-4 microplate reader (Biotek instrument) at 360 nm and 460 nm for excitation and emission wavelengths, respectively. Instrument calibration was achieved with blank solution (composed of the reaction media only) and standard additions of NADH at 50 µM in the reaction media.

2.3. Exposure to selected chemicals

The above reactions (NADH oxidase and oscillations) were studied in the presence of the following 7 chemicals: nicotine (Nic), isoniazid (Iso), erbium chloride (Er), lutetium chloride (Lu), 50 nm-diameter polystyrene nanoplastic (NPs), ibuprofen (Ibu) and 50 nm-diameter zinc oxide nanoparticles (nZnO). These chemicals are of importance because they are suspected contaminants released by municipal wastewaters [15-17]. The physical chemical properties are reported in Table 1. Most chemicals were obtained from Sigma Chemical Company with the exception of polystyrene NPs (Polysciences, USA). Polystyrene NPs and zinc oxide nanoparticles were prepared in MilliQ water at 1 mg/mL to prevent aggregation [17, 18]. The NPs and nZnO size and aggregation potential were determined by Dynamic Light Scaterring analysis (532 nm laser, Mobius Instrument, Wyatt Technologies, Santa Barbara, CA, USA) in MilliQ and tap water and revealed no aggregation and changes in the Zeta potential after 1 h dissolution. The size diameter for 50 nm NPs was confirmed at 53 ± 2 nm. The controls received the same amount of MilliQ water for nanoparticles and rare earth elements or phosphate buffered saline (140 mM NaCl, 5 mM KH_2PO_4, pH 7.4) for the organic compounds. Mitochondria were thus exposed to concentrations of Nic (0.08, 0.16, 0.24, 0.32, 0.4, 0.48 and 0.56 ug/mL),

Table 1. List of chemicals tested.

Chemicals	Molecular formula	Molecular weight/density (g/mol or particle density)	CAS number
Nicotine (+/-)	$C_{10}H_{14}N_2$	162.23	22083-74-5
Isoniazid	$C_6H_7N_3O$	137.14	54-85-3
Ibuprofen (sodium salt)	$C_{13}H_{17}O_2Na$	228.26	31121-93-4
Erbium	$ErCl_3$	273.62	10138-41-7
Lutetium	$LuCl_3$	281.33	10099-66-8
Polystyrene Nanoplastics	Polystyrene	2.5% (w/v) solids 50 nm mean diameter 3.64×10^{14} particles/mL	9003-53-6 (polystryrene)
Zinc oxide nanoparticle	ZnO	20% (w/v)as ZnO 50 nm mean diameter	1314-13-2

Iso (same as Nic), Er (1.6, 3.2, 2.5 ug/mL), Lu (same as Er), Ibu (same as Nic), nZnO (0.5, 1, 1.5, 2, 2.5 ug/mL) and polystyrene NPs (0.1, 1, 5 mg/L) *in vitro*. These concentrations were chosen for screening effects in an exploratory manner and does not necessarily represent environmental concentrations. Increasing concentration of the chemical was prepared in the incubation medium 5 min prior to the addition of mitochondria suspension. After 1 min mixing, the reactions (NADH oxidase and oscillations) were initiated in dark microplates and immediately processed by the microplate reader. Fluorescence measurements were taken each 30 sec for times up to 45 min, generating time series with 90 data points.

2.4. Data analysis

The experiments were repeated 3 times and the samples were analyzed in duplicates. The NADH oxidase activity and fractal dimension (fD) data were analyzed using non-parametric analysis of variance using the Conover-Inman non-parametric test to find differences from controls. Significance was set at $p < 0.05$. The oscillatory changes in NADH levels were first analyzed using Fourier transformation to determine the amplitude changes (periodogram) in the frequency domain. The fractal power decay of frequency was analysed using Fourier transformation of the NADH levels with time. This analysis provides the periodogram value to obtain the square amplitude changes for each frequency. The fractal parameter β from relationship log [squared amplitude] = β log (frequency) was determined. The β factor is related to the fD of media. The fD was determined by the log-log relationship between the amplitudes and frequency changes, which is considered as one of the most precise method for fD [19]. The fD was obtained from the fractal behavior of amplitude changes in the frequency domain and was obtained as follows: square Amplitudes ~ $1/f^{\beta}$ where the β exponent corresponds to the slope between the square amplitudes (y) and frequency (x) on a log-log plot. The β slope is related to the fD: $fD = (3-\beta)/2$. In other words, the fractal behaviour of wave responses consists in the appearance of scales in the frequencies where a decrease in the fD is associated to increased frequency at lower amplitudes.

3. RESULTS AND DISCUSSION

Mitochondria were used to examine the toxicity of selected environmental contaminants including plastic NPs. They respond to nicotinic receptors involved in the prevention of transition pores liberating low molecular weight organic anions. Hence, Nic and Iso compounds served as positive controls in monitoring changes in the fractal properties of mitochondria activity. This response prevents the initiation of apoptosis from mitochondria swelling. The activity of NADH oxidase activity and changes in the fD were examined (Figure 1A and B). NADH oxidase activity was significantly increased by Nic and Iso at a threshold concentration of 0.16 µg/mL. However, Nic increased the NADH oxidase activity up to 5 times the controls while Iso increased the activity by 2.2 times the controls (Figure 1A). The fD was calculated based on the rate of decrease in NADH in time and long-term oscillations which, revealed a concentration dependent decrease in the fD for NIC and Iso (Figure 1B). For Nic, a significant correlation between NADH oxidase and fD was obtained ($r = 0.63$; $p < 0.01$). For Iso, a significant correlation was also obtained between NADH and fD ($r = 0.57$; $p < 0.01$). This suggests that the reduction in the fD is associated with increased activity in NADH oxidase activity in keeping with the activation of nicotinic ACh receptors involved in the regulation of Ca^{2+} entry in mitochondria. The activation of nicotine Ach receptor prevents accumulation of Ca^{2+} and mitochondria swelling. However, increased NADH oxidation is coupled to the production of reactive oxygen species, which uncontrolled, could lead to mitochondria damage by Ca^{2+} accumulation, swelling and apoptosis [2]. These results are in keeping with the increase in mitochondria electron transport activity by cotinine-the metabolite of Nic [20] coupled to NADH oxidation. Moreover, cotinine was able to reduce lipid peroxidation in mitochondria *in vitro* suggesting that cotinine has some potential to catch ROS.

The influence of the rare earths Er and Lu on NADH oxidation rates and fD was examined. Er and Lu are found in technological devices at solid waste disposal sites where leachates could reach water bodies sometimes not connected to municipal wastewater treatment plants. Neither Er nor Lu increased the NADH oxidation rate (Figure 2A). The fD was increased by Lu for most concentrations

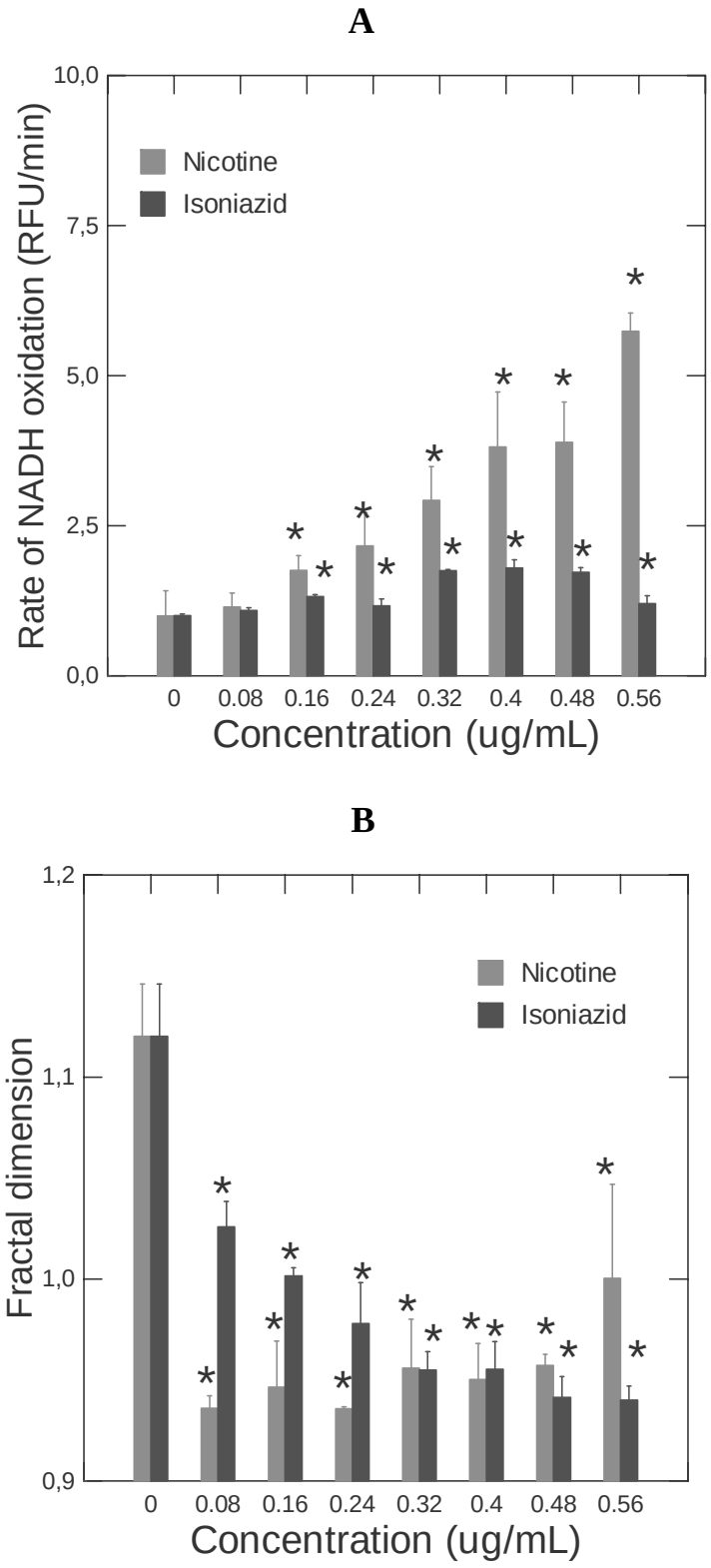

Figure 1. NADH oxidation rates and changes in the fractal dimension in mitochondria exposed to nicotine and isoniazid. The activity of NADH oxidase (**A**) and the fractal dimension fD (**B**) are shown. The data represents the mean with the standard error. The star symbol * indicates significance at $p < 0.05$. RFU: relative fluorescence units.

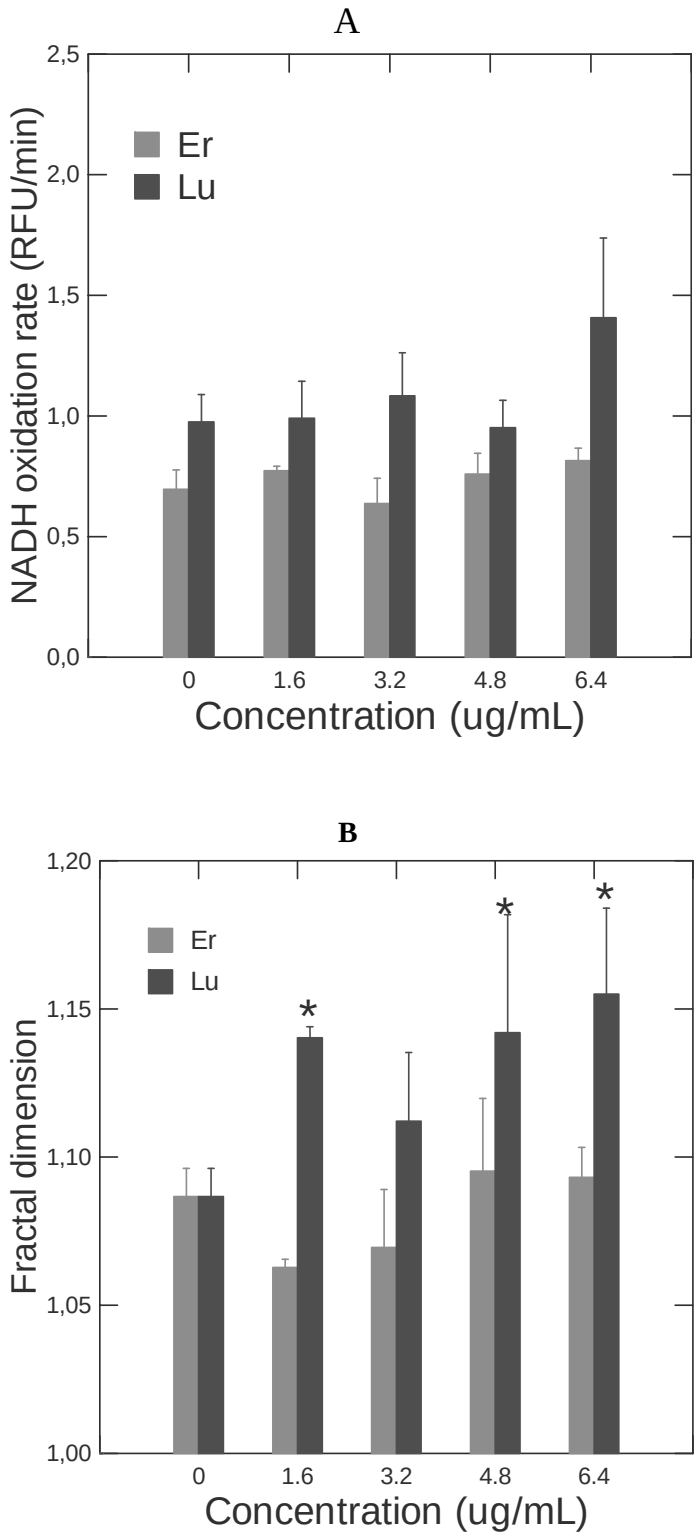

Figure 2. NADH oxidation rates and changes in the fractal dimension in mitochondria exposed to rare earths. Mitochondria suspensions were exposed to increasing concentration of Er and Lu. NADH oxidation activity (**A**) and the fractal dimension (**B**) were determined. The data represent the mean with the standard deviation. The star symbol indicates significance relative to controls.

tested (Figure 2b). This suggests that Lu was involved in the increase in the dimension of the NADH reaction and perhaps enters mitochondria. The explanation for this is unclear for the moment given that Er and Lu share similar ionic radius, electronegativity, redox potential and molecular mass. Lu is slighty more electrogenative (1.27 vs 1.24) than Er, which could confer a more divalent "calcium-like" state and propagates more easily in mitochondria albeit the differences in electronegativity are small between Er and Lu. The effects of Ibu were also examined and revealed no significant changes in NADH oxidase activity ($p > 0.05$, not shown) but the fD were significantly reduced at 0.16 µg/mL. It was shown that Ibu does not produce oxidative stress in mitochondria but could produce mitochondria-mediated apoptosis by protein aggregation and proteasomal dysfunction [21]. Ibu was shown to prevent Ca^{2+} overload and cytochrome c release thus preventing apoptosis from denatured protein aggregates [22].

We also examined the influence of polystyrene and ZnO NPs on NADH oxidase and fD in mitochondria (Figure 3). NADH oxidation rates did not significantly change in mitochondria treated with polystyrene NPs although some increase in the reaction rates were observed (Figure 3A). The fD was significantly reduced from 1.3 to 1.24 at the highest concentration but the changes were mild (Figure 3B). It was reported that a chain-like aggregation dust fractal had an fD in the range of 1.5 while a diffusion-limited aggregation dust fractal has a fD in the range of 1.25 [23]. This suggests that mitochondria and nanoparticles occupy space similar to a diffusion-limited dust pattern and are homogenously organized in space compared to control mitochondria. A chain-like dust organization occupies less volume and is more heterogeneous. This change in the mitochondria organisation towards a diffusion-limited dust fractal restricts more space, which could influence enzyme reactions involved in this organelle. Mitochondria were shown to form networks (budding), which can precede apoptosis events [24]. Mitochondria also form dissipative structures during the cyclic changes in NADH levels thought to be associated in synchronization between mitochondria [25], which can be perturbed by contaminants such as cadmium [13].

The oscillatory changes in NADH levels by the selected contaminants of this study were also examined (Figure 4). The oscillatory changes in NADH levels are depicted in Figure 4A for mitochondria treated with Nic and controls. In control mitochondria, NADH levels oscillate with a period of around 2.5 min. In the presence of Nic, a frequency decay in amplitude changes was observed, which is characteristic with the fractal behavior of frequency changes. Indeed, exposure to Nic decreased the amplitudes with a shortening of the periods to 1-1.5 min (higher frequency). Moreover, the appearance of a large amplitude oscillation with a period over 27 min was observed when Nic was added to the reaction media. One characteristic of the behavior of frequency in a fractal environment is the occurrence of frequency changes at different scales where large amplitudes are associated to low frequency and inversely [19, 26]. This behavior is sometimes coined as the power decay of frequency in fractal environments. The fractal power decay of frequency was analysed using Fourier transformation of the NADH levels in time to obtain the fractal parameter β from relationship: log [squared amplitude] = β log (frequency). The β factor (slope) was significant ($p < 0.01$) and increased by the presence of Nic compared to control mitochondria (Figure 4B). This suggests that Nic could increase the frequency in oscillations but at the expense of the strength (power) of the changes. Changes of frequencies and amplitudes were also reported for mitochondria exposed to selected elements such as zinc oxide NP, Er and Lu in mussel mitochondria [27] and Cu, Gd and silver NPs in yeast mitochondria [28]. In general, the observed changes in frequency and amplitudes followed a fractal behavior i.e., the appearance of low intensity amplitudes at higher frequencies with NPs. The amplitudes of the first 10 frequencies, β values and the fD were determined for each elements (Table 2). The amplitudes of the first 10 frequencies were higher for nicotinic compounds Nic and Iso and lower for Ibu, Er, Lu and zinc oxide NPs. Polystyrene NPs had intermediary effects on the amplitudes. The fractal behavior was also examined with the β slope between amplitude changes and frequency. The analysis revealed that the following compounds Nic, Iso, polystyrene NPs generally increased the β slope which suggests the formation of low amplitude-high frequency changes

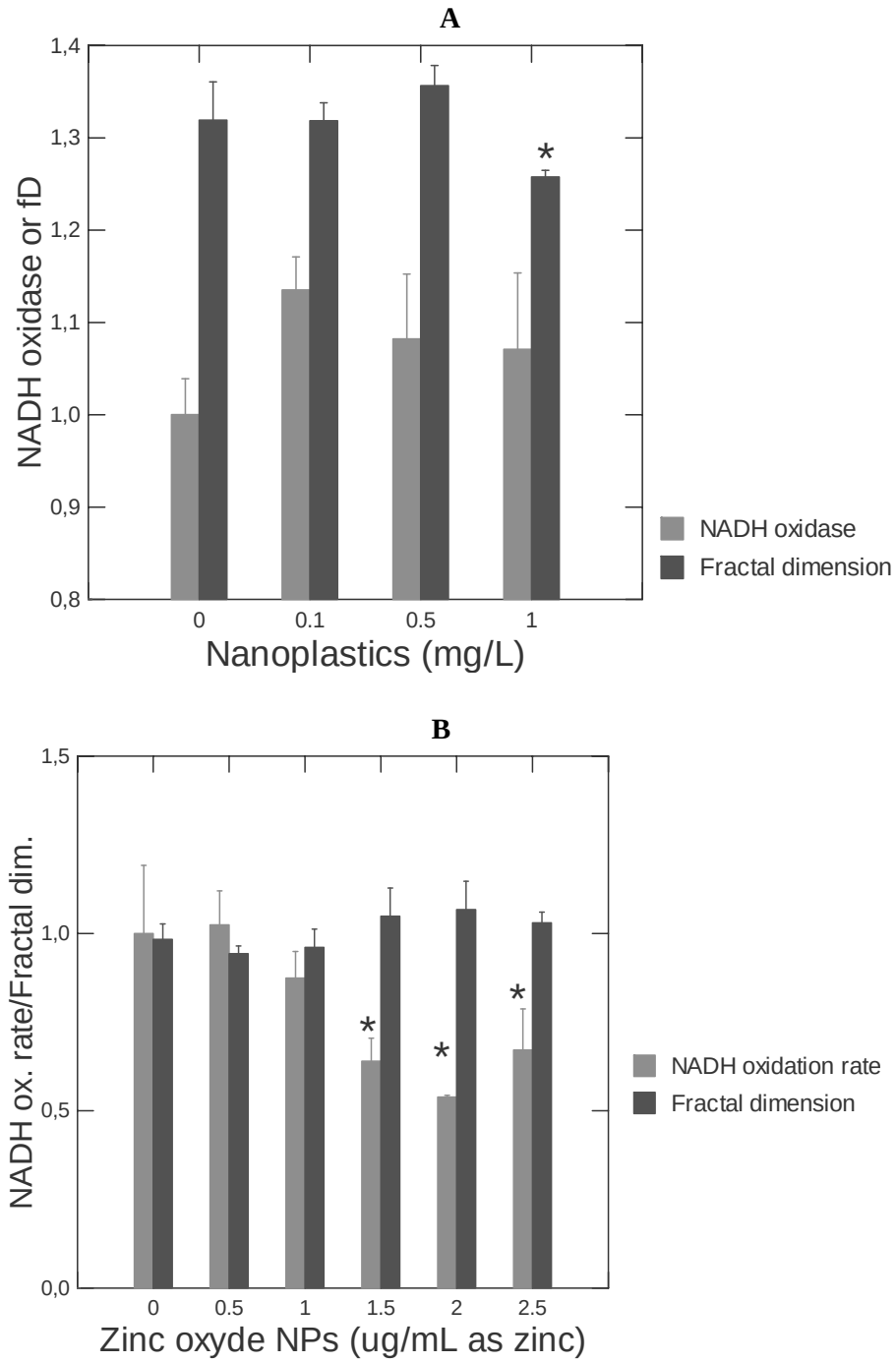

Figure 3. NADH oxidation rates in mitochondria exposed to nanoparticles. Mitochondria suspensions were exposed to increasing concentration of polystyrene (**A**) and zinc oxide (**B**) NPs. The data represent the mean with the standard deviation. The star symbol indicates significance relative to controls.

usually associated with fractals. The fD dimension was correspondingly decreased with these compounds which suggests a more crowded environment in the mitochondria suspension. A significant linear trend was obtained between the amplitude changes and the β slope (r = 0.62) or fD (r = -0.58) (Figure 5). Polystyrene NPs reduced the fD (or increase the slope β) as with the nicotinic agents Nic and Iso but

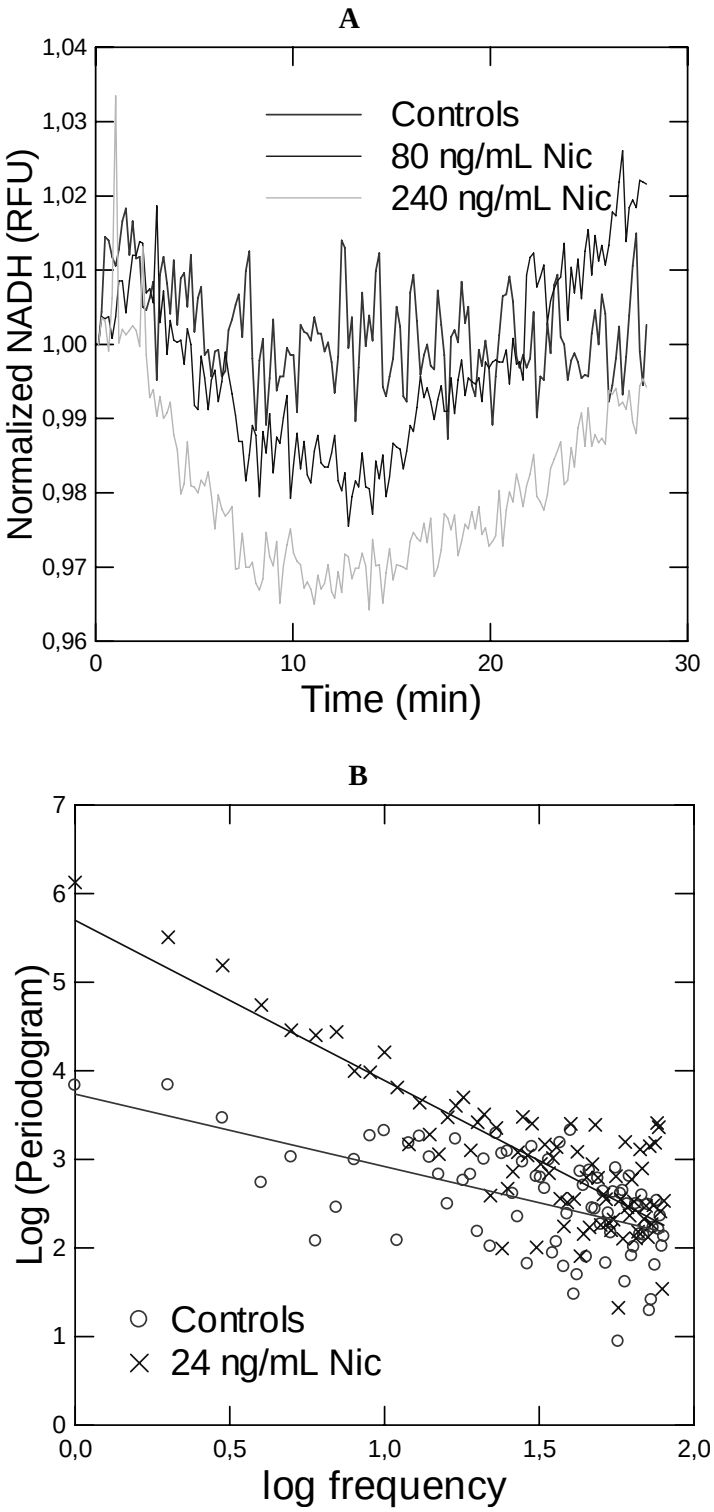

Figure 4. Effects on the oscillatory behavior in NADH levels in mitochondria exposed to nicotine. NADH levels were determined in mitochondria suspension alone and in the presence of nicotine (**A**). Note the appearance of low frequency and high amplitude changes in NADH levels (characteristic of fractal behavior of oscillations). Fractal frequency analysis of NADH changes in mitochondria alone and in the presence of nicotine (**B**). The slope corresponds to the fractal exponent β which is related to the fractal dimension of the frequency and amplitude changes: fD = (3- β)/2.

Table 2. Spectral and fractal characteristics of NADH oscillations.

Compounds	Frequency (first ten frequency)	Periodogram Normalized to controls	Fractal behavior β; fD
Controls		Sum = 10	-0.522 ± 0.05; 1.24 ± 0.09
Nicotine	0.0125	**132**	-1.106 ± 0.1*; 0.95 ± 0.05*
	0.025	4.8	
	0.075	3	
	0.1	0.5	
	0.115	1.2	
	0.125	**9.7**	
	0.15	**16**	
	0.175	0.9	
	0.20	0.1	
	0.2125	0.8	
		Sum = 151	
Isoniazid	0.0125	**65**	-1.154 ± 0.1*; 0.923 ± 0.09*
	0.025	**8.6**	
	0.075	5.4	
	0.1	3.1	
	0.115	**6.3**	
	0.125	5.2	
	0.15	3.5	
	0.175	3.8	
	0.20	5.1	
	0.2125	3.3	
		Sum = 187	
Ibuprofen	0.0125	0.4	-0.473 ± 0.05; 1.26 ± 0.1
	0.025	0.1	
	0.075	1.3	
	0.1	0.6	
	0.115	**5.6**	
	0.125	2.9	
	0.15	0.1	
	0.175	**25**	
	0.20	**5.6**	
	0.2125	0.4	
		Sum = 42	

Table 2 continued..

Naphthenic acids	0.0125	0.1	-0.20(NS)*; 1.49 ± 0.12
	0.025	0.2	
	0.075	0.2	
	0.1	**2.7**	
	0.115	0.4	
	0.125	**4**	
	0.15	0.2	
	0.175	1.4	
	0.20	1.2	
	0.2125	**6**	
		Sum = 16.4	
Er	0.0125	0.5	-0.550 ± 0.04; 1.225 ± 0.1
	0.025	0.6	
	0.075	1.6	
	0.1	**4.3**	
	0.115	**6**	
	0.125	2	
	0.15	**27**	
	0.175	0.7	
	0.20	1.7	
	0.2125	1.6 Sum = 46	
Lu	0.0125	0.3	-0.957 ± 0.1*; 1.02 ± 0.1*
	0.025	1.5	
	0.075	0.7	
	0.1	**3.4**	
	0.115	4	
	0.125	0.7	
	0.15	**10**	
	0.175	0.6	
	0.20	1.8	
	0.2125	**6**	
		Sum = 29	
Nanoplastic 50 nm	0.0125	0.1	-1.175 ± 0.2*; 0.915 ± 0.09*
	0.025	3.4	
	0.075	0.01	
	0.1	**3.6**	
	0.115	0.6	
	0.125	0.6	
	0.15	**3.7**	
	0.175	**67**	
	0.20	0.6	
	0.2125	3.1	
		Sum = 83	

Table 2 continued..

Zinc oxide nanoparticles (100 nm)	0.0125	0.2	-1.178 0.92;
	0.025	1	
	0.075	0.2	
	0.1	0.6	
	0.115	1	
	0.125	0.9	
	0.15	0.001	
	0.175	0.004	
	0.20	0.008	
	0.2125	**2.5**	
		Sum = 6.4	

1. The β exponent is defined by the slope between the square amplitudes and the frequencies in a log-log scale i.e., increased amplitudes are significantly associated with lower frequencies.

with not the same intensity. The other compounds produced less change in the fD (Er, Lu, Ibu and zinc oxide NPs). This suggests that these NPs could alter the space properties involved in the transport of high energy electrons in mitochondria. The reduction of the fD and increased NADH oxidase activity could increase the concentration of reactive oxygen species because most reactive oxygen species (ROS) are produced from mitochondria NADH oxidase [2]. This hypothesis is supported by studies showing oxidative stress and damage from nanoplastics in aquatic organisms. For example, polystyrene NPs induce ROS production in Daphnia pulex [29] (Liu *et al.*, 2020). In addition, antioxidant enzymes catalase, total and Cu/Zn superoxide dismutase were inhibited with increasing concentration of NPs, which resulted in reduced growth, development and reproduction. Exposure of hydra to 50 and 100 nm polystyrene NPs also resulted in oxidative damage [17]. Exposure to both sizes of polystyrene NPs resulted in increased lipid peroxidation, biomass and the formation of liquid crystals at the post-mitochondrial fractions. The liquid crystal state in the subcellular environment was associated with increased polar lipids levels and viscosity. The ingestion of polystyrene NPs in nematode *Caenorhabditis elegans* led to severe production of ROS in the intestine and suppression of Mn-superoxide dismutase normally associated to the aging process [30]. Interestingly, the mitochondrial unfolded protein response was suppressed by these plastic NPs. The suppression of mitochondrial protein unfolded response is associated to oxidative stress and decreasing lifespan in nematodes. Based on these results, enzyme activity could be adjusted with the fD to better understand the spatial changes in mitochondria activity. A decrease in the fD could lead to local increase in the concentrations of the substrates and products, which could influence enzyme activity at different substrate concentrations. For example, at low substrate concentrations, a decrease in the fD could lead to a local increase in the substrate concentration and increased enzyme activity [31]. Enzyme activities are usually derived in initial conditions where substrates are at saturating levels and the reaction rates constant. However this does not take into account the fractal properties of enzymes and proteins in complex biological preparations. The reaction rates in these conditions could be adjusted with the fD to have a better understanding on the context from which enzyme reactions takes place. Given that fD is derived from power law equations, enzyme activities could be "adjusted" with the sD in the following manner: $v_{fD} = v^{2/fD}$ where vfD is the adjusted rate of reaction, v the rate obtained in the initial conditions and fD the spectral dimension. If the reaction rates occurs in uncrowded environments where normal diffusion governs the rates ($fD \rightarrow D = 2$) then the mass action condition is met and $v_{fD} = v^{2/2} = v^1$ (no changes in the reaction rate v). Conversely, the reaction speed will increase locally as the fD

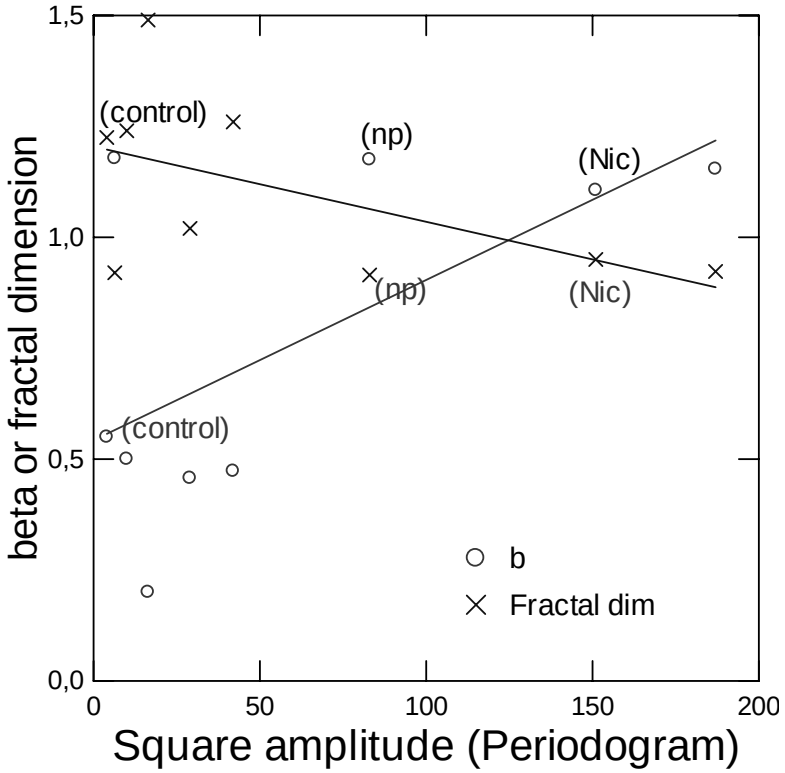

Figure 5. Relationship between the amplitudes, β exponent and the fractal dimension. The amplitudes are inversely related to the fractal dimension (r = -0.58) and directly related to the β exponent (r = 0.62) between the amplitudes and frequency range. A diminution of the fractal dimension will lead to increased amplitudes at low frequency. Polystyrene nanoparticles (np) produce effects similar to nicotine.

decreases because the local concentrations increase as the space decreases. In the case of mitochondria treated to polystyrene NPs, the adjusted rate becomes significant at the highest concentration (1 mg/L) of NPs. In other words, we could state that the global activity is not increased but is locally (and significantly) induced.

CONCLUSION

In conclusion, mitochondria exposed to nicotinic agents led to reduced fD associated to mitochondria compactness. This was accompanied with increased mitochondria NADH oxidase activity involved in the main production of ROS which, if uncontrolled could damage mitochondria. These nicotinic compounds also disrupt the normal oscillations in NADH levels, which reflects the balance between NADH production (citric acid cycle) and NADH oxidation during the electron transport step (complex 1) for ATP synthesis. The changes follow a pattern consistent with fractal organization i.e., the appearance of low amplitude changes at higher frequency in NADH levels. Exposure to polystyrene NPs produced more important changes compared to Er, Lu, Ibu and zinc oxide NPs suggesting that the crowding of NPs could reduce the spatial (fractal) dimensions of mitochondria. Reductions in the fD of NADH oxidation in mitochondria could represent a mechanism of oxidative stress caused by plastic NPs. The analysis of the fractal kinetics of mitochondria activity provides information on the effects of various environmental compounds on the spatial organization of critical life-supporting biochemical processes.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest associated with this publication.

REFERENCES

1. Aon, M. A., Cortassa, S. and O'Rourke, B. 2008, Adv. Exp. Med. Biol., 641, 98.
2. Vinogradov, A. D. and Grivennikova, V. G. 2016, Biochim. Biophys. Acta, 1857, 863.
3. Giorgi, C., Marchi, S., Simoes, I. C. M., Ren, Z., Morciano, G., Perrone, M., Patalas-Krawczyk, P., Borchard, S., Jędrak, P., Pierzynowska, K., Szymański, J., Wang, D. Q., Portincasa, P., Węgrzyn, G., Zischka, H., Dobrzyn, P., Bonora, M., Duszynski, J., Rimessi, A., Karkucinska-Wieckowska, A., Dobrzyn, A., Szabadkai, G., Zavan, B., Oliveira, P. J., Sardao, V. A., Pinton, P. and Wieckowski, M. R. 2018, Int. Rev. Cell. Mol. Biol., 340, 209.
4. Meyer, J. N., Leung, M. C. K., Rooney, J. P., Sendoel, A., Hengartner, M. O., Kisby, G. E. and Bess, A. S. 2013, Toxicol. Sci., 134, 1.
5. Malińska, D., Więckowski, M. R., Michalska, B., Drabik, K., Prill, M., Patalas-Krawczyk, P., Walczak, J., Szymański, J., Mathis, C., Van der Toorn, M., Luettich, K., Hoeng, J., Peitsch, M. C., Duszyński, J. and Szczepanowska, J. 2019, J. Bioenerg. Biomem., 51, 259.
6. Bhadauria, S., Mishra, R., Kanchan, R., Tripathi, C., Srivastava, A., Tiwari, A. and Sharma, S. 2010, Toxicol. Mech. Meth., 20, 242.
7. Skok, M., Gergalova, G., Lykhmus, O., Kalashnyk, O., Koval, L. and Uspenska, K. 2016, Neurotransmitter, 3, e1290.
8. Klamerth, N., Malato, S., Maldonado, M. I., Agüera, A. and Fernández-Alba, A. R. 2010, Environ. Sci. Technol., 44, 1792.
9. Sakamuru, S., Attene-Ramos, M. S. and Xia, M. 2016, Methods Mol. Biol., 1473, 17.
10. Roubicek, D. A. and Souza-Pinto, N. C. 2017, Toxicol., 391, 100.
11. Lloyd, D., Murray, D. B., Aon, M. A., Cortassa, S., Roussel, M. R., Beckmann, M. and Poole, R. K. 2018, J. Biomed. Opt., 24, 1-17.
12. MacDonald, M. J., Fahien, L. A., Buss, J. D., Hasan, N. M., Fallon, M. J. and Kendrick, M. A. 2003, The J. Biol. Chem., 278, 51894.
13. Hanana, H., Kleinert, C., André, C. and Gagné, F. 2019, Comp. Biochem. Physiol., 216C, 60-66.
14. Bradford, M. M. 1976, Anal. Biochem., 72, 248.
15. Li, Z., Zheng, T., Li, M. and Liu, X. 2018, Environ. Sci. Poll. Res. Int., 25, 26852.
16. Metcalfe, C. D., Miao, X. S., Koenig, B. G. and Struger, J. 2003, Environ. Toxicol. Chem., 22, 2881.
17. Auclair, J., Quinn, B., Peyrot, C., Wilkinson, K. J. and Gagné, F. 2020, Environ. Sci. Poll. Res. Int., 27, 11772.
18. Gagné, F., Auclair, J., Turcotte, P., Gagnon, C., Peyrot, C. and Wilkinson, K. 2019, Comp. Biochem. Physiol. C Toxicol. Pharmacol., 219, 1.
19. Schepers, H. E., van Beek, J. H. G. and Bassingthwaighte, J. B. 2002. IEEE Eng. Med. Biol. Mag., 11, 57.
20. Gagné, F., Blaise, C., André, C. and Salazar, M. 2006, Comp. Biochem. Physiol. C Toxicol. Pharmacol., 143, 388.
21. Upadhyay, A., Amanullah, A., Chhangani, D., Joshi, V., Mishra, R. and Mishra, A. 2016, Mol. Neurobiol., 53, 6968.
22. Sanz-Blasco, S., Valero, R. A., Rodríguez-Crespo, I., Villalobos, C. and Núñez, L. 2008, PLoS One, 23, e2718.
23. Kopelman, R., 1986, J. Stat. Phys., 42, 185.
24. Haga, N., Fujita, N. and Tsuruo, T. 2003, Oncogene, 22, 5579.
25. Holmuhamedov, E. L. 1986, Eur. J. Biochem., 158, 543.
26. Bies, A. J., Boydston, C. R., Taylor, R. P. and Sereno, M. E. 2016, Symmetry, 66, 1.
27. Gagné, F. and Hanana, H. 2018, J. Anal. Toxicol. Appl., 2, 7.
28. André, C. and Gagné, F. 2017, Comp. Biochem. Physiol., 196, 36.
29. Liu, Z., Huang, Y., Jiao, Y., Chen, Q., Wu, D., Yu, P., Li, Y., Cai, M. and Zhao, Y. 2020, Aquat. Toxicol., 220, 105420.
30. Qiu, Y., Luo, L., Yang, Y., Kong, Y., Li, Y. and Wang, D. 2020, Sci. Total Environ., 705, 135918.
31. Sharma, A., Shinde, U. P. and Kulkarni, B. D. 1990, Biotechn. Let., 12, 737.