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The Effects of Magnesium Oxide Nanoparticles on Population Growth of *Paramecium caudatum*

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ABSTRACT

Physicochemical properties of materials have been changed at nanoscale. It is so considerable for scientists and researchers to investigate and analyze the properties of nanomaterials compared with conventional ones. On the other hand, protozoa have been considered as important model organisms for biological studies and research activities**.** In this regard, in addition to the ecological and environmental importance, the population growth rate of these microorganisms is significant for laboratory researches. In this research, about 48 hours after culture, Paramecia cells were observed, via the 4× light microscope objective lens, and counted by Sedgewick-Rafter counting chamber. Then, the effects of Ca^{2+}, Mg^{2+} and magnesium oxide nanoparticle (MgO NP) on the population growth of *Paramecium caudatum* were analyzed. According to results, the highest population growth has been obtained in the case of yeast medium enrichment using Ca^{2+} +MgO NP compared with $CaCl_2$+$MgCl_2$ ($p < 0.01$). These findings have demonstrated that enriched yeast medium with MgO NP and $CaCl_2$ is one of the most appropriate specific media for culture and proliferation of *P. caudatum* leading to easy and frequent access to abundant Paramecia cells for laboratory research activities emphasizing the ecological and environmental importance of this sensitive microorganism.

Keywords: *Paramecium caudatum*; Population growth; Magnesium oxide nanoparticle; Enriched yeast medium

INTRODUCTION

Physicochemical properties of materials have been changed at nanoscale due to decreasing the size and increasing the number of particles at nanoscale. It means that more contact surface leads to increasing the contacts of molecules and velocity of chemical reactions. Therefore, it is so important for scientists and researchers to investigate and analyze the properties of nanomaterials compared with conventional ones. It can provide a new approach in utilization of nanomaterials. Since in biological systems, physicochemical effects appear at the cellular and molecular levels, it seems that nanomaterials have more significant effects than conventional ones in living things. Meantime, many physiological investigations have been performed on the effects and functions of some bioelements such

as magnesium, zinc, iron, etc., in the form of nano, in biological systems and physiological structures of living organisms (Torabi et al. 2013; Yang et al. 2013; Sargholi et al. 2015; Kesmati et al. 2016; Hagens et al. 2007). Therefore, the comparison of nanomaterials with conventional ones can help to solve the problems and unknown aspects of pure and applied biological science significantly.

On the other hand, protozoa, a group of animal-like microorganisms composed of a cell, comprise a large group namely ciliata with cosmopolitan distributions living in almost all aquatic environments such as swamps, seas, lakes, springs and soils. Since the protozoa as the organisms which are sources of living things consist of simple and unicellular structures, they have been considered as important model organisms for biological studies and researches such as cell physiology (Montagnes et al. 2012; Warren 2013). In this regard, a ciliated protozoan, *Paramecium caudatum* is very sensitive to environmental changes, and the circumstances like concentration of ions such as Ca^{2+} and Mg^{2+} have significant impacts on population growth rate of this unicellular animal-like microorganism (Laybourn-Parry 1984; Prajer et al. 1997; Shahrokhi et al. 2013). In addition to the ecological and environmental importance, the population growth rate, as an important physiological factor, of this initial animal-like microorganism is significant for laboratory research activities (Montagnes et al. 2012; Warren 2013). So, brief descriptions of the above issues seem to be necessary here:

First: Ciliata are the primary consumers and decomposers in ecosystem. They play an important role in the cycle of matter and energy (Adl and Gupta, 2006).

Second: These protozoa are the environment purifiers as Bacterivore and Saprovore converting the materials not absorbed by plants to absorbable ones; such that plants will absorb these materials directly or indirectly. This can be effective in the forest preservation and restoration by ciliata (Adl and Gupta, 2006), as well as very important in municipal and industrial wastewater purification (Curds and Cockburn, 1970a; Curds and Cockburn, 1970b; Curds 1973; Curds 1975).

Third: *P. caudatum* can be used as a model organism, because of high proliferation potential, relatively large size, accessibility and ease of culture in the laboratory. So, in order to use this model organism in laboratory research activities, the optimum conditions and appropriate concentrations of required materials and ions should be provided for better culture and proliferation of this unicellular organism, for example in this work *P. caudatum* is cultivated in a specific medium. This enriched medium is prepared by means of gradients and concentrations described in the materials and methods (Montagnes et al. 2012; Shahrokhi et al. 2013; Warren 2013).

Although there are many scientific reports and research papers about the toxicity of nanoparticles on ciliated protozoa, particularly, the effect of nanoparticles on the increase of population growth of these microorganisms has not ever been investigated. Therefore, in this research, the effect of magnesium oxide nanoparticle (MgO NP) compared with $CaCl_2$

(Ca^{2+}), conventional magnesium oxide (cMgO) and $MgCl_2$ (Mg^{2+}) on the population growth of *P. caudatum*, as an important physiological factor, has been investigated in specific culture media such as yeast medium. Because the Ca^{2+} and Mg^{2+} play important roles in the process of cell division (Prajer et al. 1997; Malvin et al. 2003; Shahrokhi et al. 2013).

MATERIALS AND METHODS

Sampling, identification and cultivation

In our previous research, sampling and morphological identification of *P. caudatum* have been performed; as well, cultivation of this microorganism has been conducted in natural and specific media in the laboratory until now (Karami et al. 2013a; Karami et al. 2013b; Shahrokhi et al. 2013).

Molecular identification

To confirm paramecium species, PCR analysis was done to detect the presence of the species specific mitochondrial small subunit ribosomal RNA (mtrRNA) gene applying the primers (Bioneer, South Korea) used in the reaction, forward primer Pa-14628F with the sequence 5'- ACC TTG TGT CAA CTT CAC TCC GAT CAT T -3' and reverse primer Pa-15257R with the sequence 5'- GGT ATT GCA TGG CTG TCG TCA GTT -3'(Przyboś and Tarcz, 2015). Then, amplified fragments corresponding to the size predicted for Paramecium species were purified using a PCR purification kit (Vivantis, Malaysia). The purified products were sequenced using an external sequencing core service (Macrogen Inc., World Meridian Center 908, 60–24 Gasan-dong, Gumchungu Seoul, Korea). Afterward, sequences were analyzed using Chromas 2.13 software and aligned with published mtrRNA gene sequences of the different Paramecium species by the GenBank nBlast program. Finally, comparison of mtrRNA sequences of the Paramecium isolate examined in the present study with those of Paramecium species from GenBank confirmed that the isolate analyzed belonged to the single species *Paramecium caudatum* with 98% similarity.

Optimum cultivation conditions

P. caudatum was cultivated in fresh specific medium containing *Saccharomyces cerevisiae*, a species of budding yeast (2 g/l of sterile distilled water, that was autoclaved at 115°C for 10 min followed by fast cooling), which was enriched by salts (0.2 g/l $CaCl_2$ and 0.2 g/l $MgCl_2$).This medium was adjusted to a pH of 6.8 ± 0.2 and was maintained at 29 to 31°C (Malvin et al. 2003; Shahrokhi et al. 2013).

Materials

Magnesium Oxide Nanoparticle (MgO NP) was purchased from Lolitech Co., Germany (particle size <50 nm), MgO NP suspensions were prepared by sonication for 15 min in ultrasonic bath before each treatment. Conventional Magnesium Oxide (cMgO) and the other chemicals ($CaCl_2$, $MgCl_2$) were prepared from Merck chemicals (Germany).

Counting cell population of Paramecia

Counting was performed using Sedgewick-Rafter counting chamber (Graticules, Ltd., UK). This chamber holds a little more than 1 ml. Initially the microorganisms (Paramecia cells) were cultured and treated (by the materials mentioned above) in vessels, each of which, contained 100 ml of culture medium. About 48 h after culture, 1 ml of culture medium was transferred into the chamber. Then, cells were observed, via the 4× light microscope objective lens, and counted; data were also simultaneously recorded (Shahrokhi et al. 2013).

Statistical analysis

Paramecia cells counting was repeated 5 times (carried out in 5 separate vessels, n = 5). At first, data were examined with Kolmogorov-Smirnov (K.S.) test, representing normal distribution. Then, data were analyzed with one-way ANOVA. Post-Hoc tests such as Tukey and LSD were utilized to realize the differences among related groups considering the alpha coefficient of 0.05 ($p < 0.05$).

RESULTS

The effect of Ca^{2+} ($CaCl_2$)

The effects of Ca^{2+} on population growth of *P. caudatum* and related data were analyzed. According to the test results: ($F(5, 24) = 27.123$, $p < 0.001$) as referred to Fig. 1, significant differences were observed at doses of 0.1, 0.2 and 0.4 mg/ml compared with the control (no treatment); such that 48 h after the culture (see the Material and Methods section) more increase in the population growth of *P. caudatum* was indicated at dose of 0.2 mg /ml $CaCl_2$ in a dose-dependent manner.

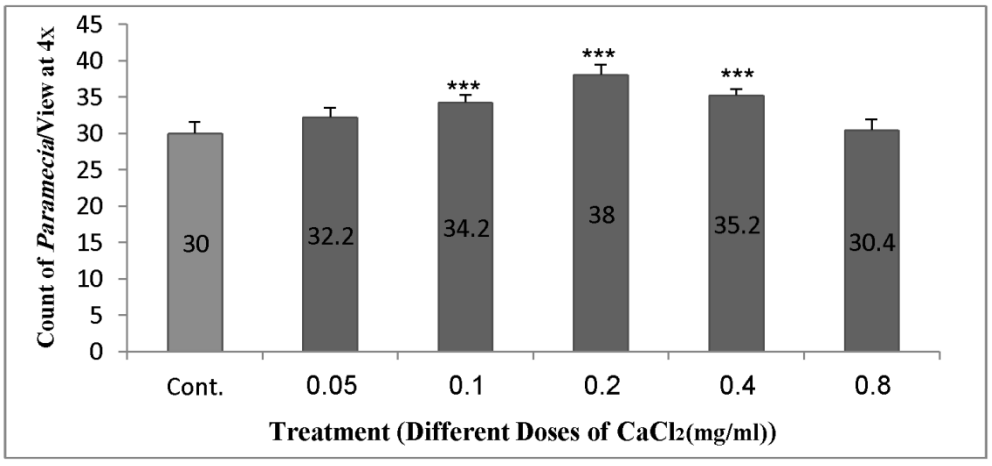

Fig. 1. The number of Paramecia cells at view 4×, 48 h after culture (and treatment) at 30° C in yeast medium with details discussed in the Material and Methods. Cells received different doses of $CaCl_2$ (0.05 to 0.8 mg/ml). *** Indicates $p < 0.001$ compared with control (no treatment). Data shown as Mean ± SD.

The effect of Mg^{2+} ($MgCl_2$)

Data on Mg^{2+} were statistically analyzed. As shown in Fig. 2, significant differences were observed at doses of 0.1 and 0.2 mg/ml compared with the control ($F(5, 24) = 6.668$, $p < 0.01$); the results revealed that at dose of 0.2 mg /ml $MgCl_2$, more increase occurred in population growth of *P. caudatum*.

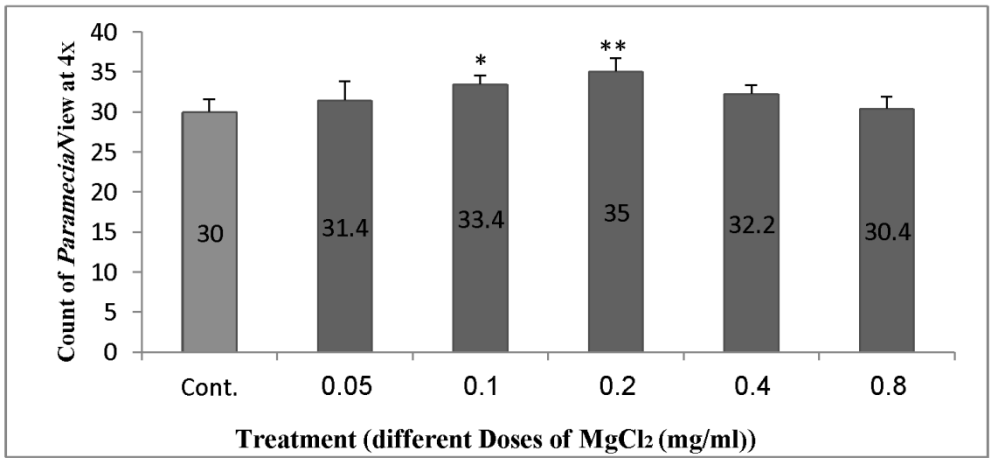

Fig. 2. Paramecia cells were treated by different doses of $MgCl_2$ (0.05 to 0.8 mg/ml) and 48 h after treatment the cells were counted (see Material & Methods). * Indicates $p < 0.05$, ** $p < 0.01$, compared with control (no treatment). Data shown as Mean ± SD.

The effect of cMgO

According to the statistical results of cMgO effect on the population growth of *P. caudatum*, no significant differences were recognized at all doses compared with the control ($F(5, 24) = 4.036$, $p < 0.01$) (Fig. 3).

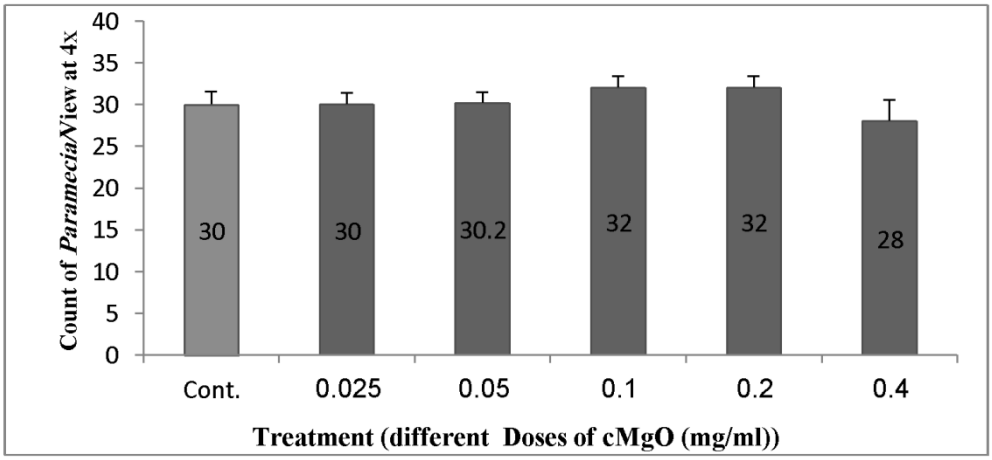

Fig. 3. Paramecia cells were treated by different doses of cMgO (0.025 to 0.4 mg/ml) and 48 h after treatment the cells were counted (see Material & Methods). no significant difference in all doses of cMgO compared with control (No treatment). Data are arithmetic Mean ± SD.

The Effect of MgO NP

The analysis results of MgO NP effect on the population growth of *P. caudatum* were also investigated. Figure 4 indicated that the differences at doses of 0.05, 0.1, 0.2 and 0.4 mg/ml MgO NP compared with the control was completely significant in a dose-dependent manner ($F(5, 24) = 498.115$, $p < 0.001$); such that, higher increase in population growth of *P. caudatum* was indicated at dose of 0.1 mg /ml MgO NP, whereas severe decrease in population growth of *P. caudatum* occurred at doses of 0.2 and 0.4 mg/ml MgO NP.

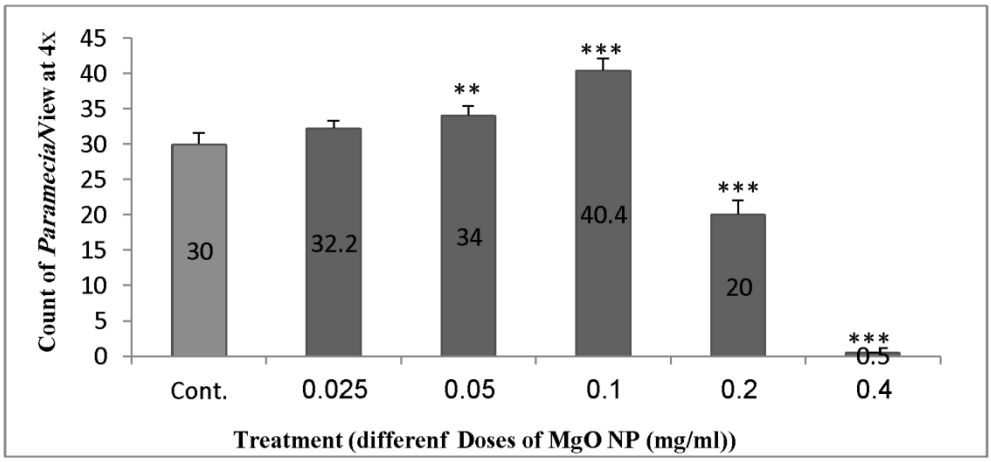

Fig. 4. Effect of MgO NP on population growth of Paramecia cells at view 4×, 48 h after culture (see Material & Methods). Cells received different doses of MgO NP (0.025 to 0.4 mg/ml). ** Indicates $p < 0.01$, *** $p < 0.001$ compared with control (no treatment). Data are arithmetic Mean ± SD.

MgO NP compared with $MgCl_2$

Data on the effects of MgO NP compared with those of $MgCl_2$ on population growth of *P. caudatum* were analyzed. Figure 5 demonstrated that significant differences at dose responses of MgO NP (0.1 mg/ml) and $MgCl_2$ (0.2 mg/ml), respectively compared with the control as well as the difference in dose response of MgO NP (0.1 mg/ml) compared with that of $MgCl_2$ (0.2 mg/ml) were observed ($F(2, 12) = 48.892$, $p < 0.001$); the results represent more increase in population growth of *P. caudatum* at dose of 0.1 mg/ml MgO NP compared with 0.2 mg/ml $MgCl_2$.

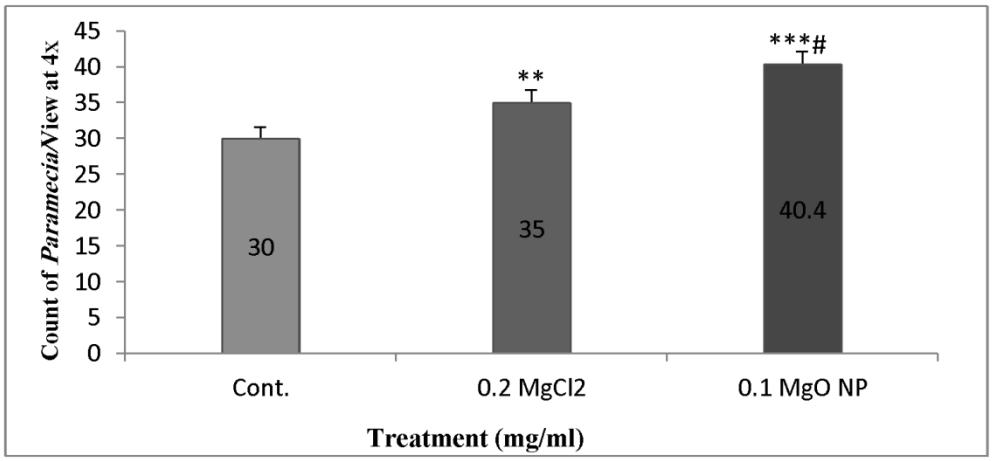

Fig. 5. Dose responses of MgO NP (0.1 mg/ml) and $MgCl_2$ (0.2 mg/ml) compared with control (no treatment) and with each other. ** Indicates p <0.01, *** p <0.001 compared with control. # indicates p < 0.01 compared with 0.2 $MgCl_2$. Data shown as Mean ± SD.

MgO NP + $CaCl_2$ compared with $CaCl_2$ + $MgCl_2$

The statistical results of MgO NP + $CaCl_2$ effect compared with those of $CaCl_2$ + $MgCl_2$ on the population growth of *P. caudatum* were analyzed. As indicated in Fig. 6, fully significant differences at dose responses of 0.1+ 0.2 mg/ml MgO NP + $CaCl_2$ and 0.2 + 0.2 mg/ml $CaCl_2$ + $MgCl_2$ compared with the control were observed ($F(2, 12) = 109.171$, $p < 0.001$); as well, the difference in these doses compared with each other showed that the highest population growth rate of *P. caudatum* was indicated at dose of 0.1 + 0.2 mg/ml MgO NP + $CaCl_2$ compared with 0.2 + 0.2 mg/ml $CaCl_2$ + $MgCl_2$.

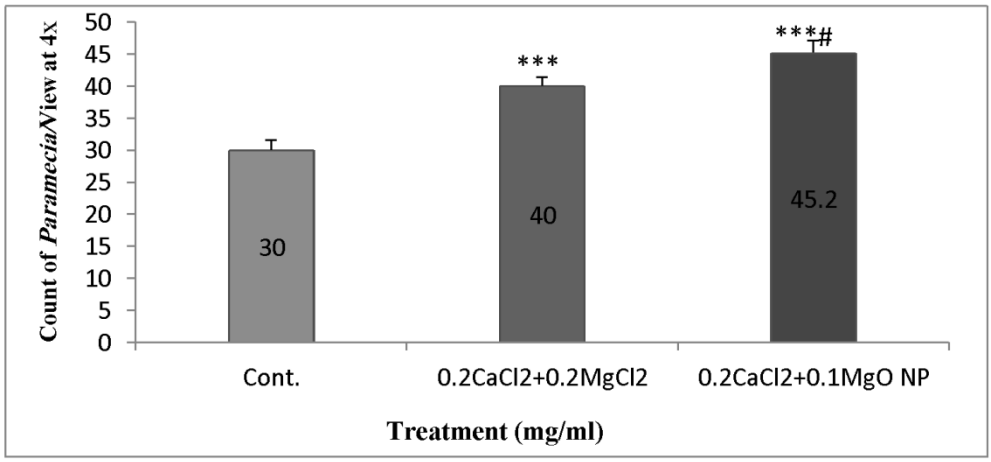

Fig. 6. Dose responses of $CaCl_2$+MgO NP (0.2 + 0.1 mg/ml) and $CaCl_2$+$MgCl_2$ (0.2 + 0.2 mg/ml) compared with control (no treatment) and with each other. *** Indicates p <0.001 compared with control. # indicates p < 0.01 compared with $CaCl_2$+$MgCl_2$ (0.2 + 0.2 mg/ml). Data are arithmetic Mean ± SD.

DISCUSSION

Ca^{2+} ($CaCl_2$) and Mg^{2+} ($MgCl_2$) each alone could make a significant increase in population growth of *P. caudatum*, with regards to more effect of Ca^{2+} which may be due to a higher share of this ion in cell division process (Prajer et al. 1997; Shahrokhi et al. 2013) (Figs. 1 and 2). But for different doses of cMgO, no significant increase has been observed in population growth of *P. caudatum* (Fig. 3), probably due to low solubility product (Ksp) of cMgO in the water. However, the greater increase in population growth of *P. caudatum* has been indicated in a dose-dependent manner at the dose of 0.1 mg/ml MgO NP compared with other doses. It is likely as a result of the nanoparticle properties it has (Fig. 4), because the more particles at nanoscale means the more contact surface leading to increase in the contacts of molecules and velocity of chemical reactions inside the cell, including cell division; such that the physiological effects of these reactions appear as population growth of *P. caudatum,* it is because the magnesium ions are essential for cell division (Walker and Duffus, 1980). In fact, this is probably resulted from changing the physicochemical properties of materials at nanoscale. But, the severe decline in population growth of *P. caudatum* has been observed at doses of 0.2 and 0.4 mg/ml MgO NP. This is probably due to toxicity of MgO NP at doses higher than 0.1 mg/ml; such that at dose of 0.4 mg/ml, no *P. caudatum* growth has been approximately observed in yeast medium, it is probably because the interaction between MgO nanoparticles and cell triggers a cascade of molecular events which could induce toxicity and cell death (Krishnamoorthy et al. 2012). Finally, the maximum population growth of *P. caudatum* with significant difference ($p < 0.01$) has been observed in the case of additive effect of MgO NP along with Ca^{2+} ($CaCl_2$) in yeast medium compared with treatments of Ca^{2+} along with Mg^{2+} ($CaCl_2$+$MgCl_2$) (Fig. 6). This highlights the important effect of MgO NP on population growth of *P. caudatum*, repeatedly. Thus, enriched yeast medium with MgO NP and $CaCl_2$, is one of the most appropriate specific media for culture and proliferation of *P. caudatum*, as a model organism, leading to easy and frequent access to abundant Paramecia for laboratory research activities (Montagnes et al. 2012; Warren 2013). Indeed, these findings confirm the extreme effectiveness of environment on this ciliated protozoan, as well the ecological and environmental importance of this sensitive and resistant microorganism (Adl and Gupta, 2006; Curds and Cockburn, 1970a; Curds and Cockburn, 1970b; Curds 1973; Curds 1975).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUBMISSION DECLARATION AND VERIFICATION

The authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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