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Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in *Daphnia magna*

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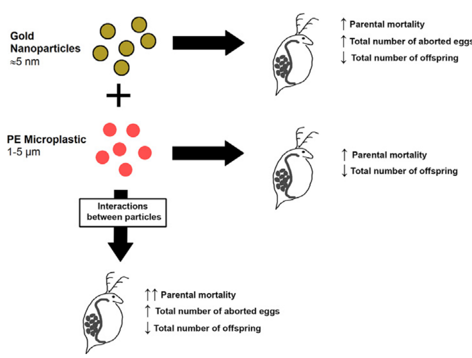
HIGHLIGHTS

- 21-day *D. magna* exposure to 5 nm AuNP caused mortality and reproduction impairment.
- 21-day *D. magna* exposure to 1–5 μm MP caused mortality and reproduction impairment.
- Mixtures of AuNP and MP caused higher effects than the components individually.
- Based on mortality, synergism at high concentrations of mixture components was found.
- Based on mortality, antagonism at low concentrations of mixture components was found.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of emerging environmental contaminants on human and environmental health is of high concern, especially those potentially induced by mixtures. The main goal of the present study was to assess the chronic effects of mixtures of citrate stabilized ≈5 nm gold nanoparticles (AuNP) and 1–5 μm microplastics (MP) on *Daphnia magna*. A 21-day bioassay was carried out. The effect criteria were parental mortality, somatic growth and several reproductive parameters. AuNP induced parental mortality, reduced the total offspring and caused immobile juveniles and aborted eggs. MP induced parental mortality, delayed the first brood release, decreased the number of broods released, the total offspring, and caused immobile juveniles. All the mixtures caused higher toxicity than AuNP and MP alone. Based on parental mortality, evidences of antagonism between AuNP and MP were observed at low concentrations of both mixture components, whereas evidences of synergism at high concentrations were found. Chronic (21-day) exposure of *D. magna* to AuNPs, MP, and their mixtures can impair development, reproduction, ultimately leading to death.

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1. Introduction

In natural habitats, organisms are commonly exposed simultaneously to several environmental contaminants (Martins et al., 2013; Lopes et al., 2016). The biological effects resulting from such exposures may be considerably different from those induced by exposure to each contaminant individually due to toxicological interactions. Current knowledge about mixture toxicity is still limited (Lopes et al., 2016; Jośko et al., 2017), especially regarding emergent contaminants of high concern such as nanomaterials and microplastics (Ferreira et al., 2016). Considering that in real scenarios the most common situation is the simultaneous exposure to mixtures rather than to single contaminants (Martins et al., 2013; Lopes et al., 2016), more knowledge is urgently needed in order to improve modelling, risk assessment, and to increase environmental and human safety under a scenario of increasing chemical use resulting from global human population and industrialization growth.

Gold nanoparticles (AuNP) are one of the most widely used nanomaterials (Khlebtsov and Dykman, 2011), mainly due to their biocompatibility and unique optical and electronic properties (Dedeh et al., 2015). In the last decades, several studies on their toxicity have been carried out. However, these studies focused on a limited variety of biological systems. In aquatic species, AuNP have been found to cause algae mortality (Renault et al., 2008; Iswarya et al., 2017), adsorb to microalgae cell walls, causing cell wall disturbances (Renault et al., 2008; Gilroy et al., 2014), accumulate in digestive epithelia of *Gammarus fossarum* (Baudrimont et al., 2017) and in the digestive tract of *D. magna* (García-Cambero et al., 2013; Khan et al., 2014, Gilroy et al., 2014; Skjolding et al., 2014; Wray and Klaine, 2015). Moreover, AuNP induced oxidative stress has been reported in *Mytilus edulis* (Tedesco et al., 2010), *Scrobicularia plana* (Pan et al., 2012) and *D. magna* (Dominguez et al., 2015). AuNP were also found to cause alterations in the expression of cellular stress genetic markers in *D. magna* (Dominguez et al., 2015), decrease the predatory performance of *Pomatoschistus microps* (Ferreira et al., 2016), among other toxic effects. Accumulation of gold in the body/tissues of aquatic organisms after exposure to AuNP (Ferreira et al., 2016) and trophic transfer from microalgae to *D. magna* were also found (Gilroy et al., 2014; Lee et al., 2015).

Microplastics (MP) are global contaminants that have been raising high concern regarding environmental and human health. They have been found to be ingested by both freshwater (Silva-Cavalcanti et al., 2016) and marine organisms (Sá et al., 2015; Phillips and Bonner, 2015; Jabeen et al., 2017). After ingestion, MP can cause a wide range of adverse effects, such as reduction of feeding activity (Ramos et al., 2012; Wright et al., 2013; Watts et al., 2015; Blarer and Burkhardt-Holm, 2016), inflammation and lysosomal membrane destabilization (von Moos et al., 2012), cholinesterase inhibition and several other (Browne et al., 2008; Oliveira et al., 2013; Au et al., 2015; Avio et al., 2015; Luís et al., 2015; Cole et al., 2015; Hu et al., 2016; Lu et al., 2016; Ferreira et al., 2016; Fonte et al., 2016; Jeong et al., 2016; Heindler et al., 2017). *D. magna* may ingest small MP (including nano sized ones known as nanoplastics) likely by passive filtration (Rosenkranz et al., 2009). After ingestion, MP can cause mortality in this species even under a short period of exposure (24–96 h) (Rehse et al., 2016; Jemec et al., 2016). In chronic exposures (21-day) to sub-lethal concentrations, reproductive fitness is impaired by some MP (Ogonowski et al., 2016).

Despite the limited number of studies investigating the presence and concentrations of AuNP in natural waters, probably because appropriate technology has only been developed recently (e.g. Long et al., 2016), these particles are believed to be common contaminants in several anthropogenic impacted areas (Teles et al., 2017; Baudrimont et al., 2017). Since MP are global contaminants and occur at increased concentrations in aquatic ecosystems of several anthropogenic impacted regions (Eriksen et al., 2013; Castañeda et al., 2014; McCormick et al., 2014; Free et al., 2014), the simultaneous exposure of aquatic organisms inhabiting these ecosystems to MP and AuNPs is likely to occur. In a recent short-term study (Ferreira et al., 2016) with the estuarine fish *Pomatoschistus microps* investigating the single and combined effects of MP and AuNP after 96 h of exposure, no significant toxicological interactions between the two types of particles on the biological parameters analysed were found. However, interactions between MP and AuNP in test media were found, which highlights the importance of further investigating the topic, particularly using long-term exposures.

The main goal of the present study was to investigate the chronic effects caused by mixtures of AuNP ($\approx$ 5 nm diameter) and plastic microspheres (1–5 μm diameter) on *Daphnia magna*, a model species widely used as representative of freshwater primary producers.

2. Material and methods

2.1. Chemicals

AuNP stabilized in citrate buffer were purchased from Sigma-Aldrich (USA), lot number: MKBP4643V. According the manufacturer description, they had a diameter between 4 and 7 nm (< 12% variability in size), a polydispersity index lower than 0.2, and a maximal spectrophotometric absorbance between 510 and 525 nm. Fluorescent red microspheres (MP) (Lot number: 4–1006-1053), were purchased from Cospheric-Innovations in Microtechnology (USA). The particles were described by the manufacturer as having between 1 and 5 μm of diameter, being red, having a density of 1.3 g/cm^3, excitation peak at 575 nm and emission peak at 607 nm. All chemicals used to prepare *D. magna* and *Chlorella vulgaris* (used as food for *D. magna*) cultures and test media were of analytical grade and purchased from Sigma-Aldrich (USA) or Merck (Germany).

2.2. Characterization of AuNP and standardization of the conditions to determine their concentrations and behaviour in test media during the bioassay

UV–vis spectrophotometry was used because is an adequate and cost-effective method to calculate the size, shape and concentrations of this type of particles in aqueous media (Haiss et al., 2007; Amendola and Meneghetti, 2009; Pamies et al., 2014). During the toxicity bioassay, the medium used was the American Society for Testing and Materials hard water (ASTM, 1980), hereafter indicated as ASTM. To investigate the suitability of this method to determine the concentrations of $\approx$ 5 nm AuNP in ASTM, six AuNP colloidal solutions (concentration of 20 mg/l) were prepared by dilution of the commercial citrate buffered AuNP solution provided by Sigma-Aldrich: three in ultra-pure (u.p.) water and three in ASTM. These solutions were then serially diluted [1:2 (*v*/v) dilution factor] in u.p. water or ASTM, according to their preparation medium, to obtain a series of colloidal solutions (hereafter indicated as solutions) with concentrations ranging from 20 to 0.039 mg/l. The UV–Vis spectra (200–900 nm) of these solutions were determined (UV–Vis, Jasco 630 spectrophotometer, USA). The surface plasmon resonance peaks (hereafter indicated as PRP) were obtained at 520 nm (Fig. S-1A), in good agreement with the literature (Amendola and Meneghetti, 2009; Yu and Andriola, 2010). To investigate possible changes in the particles through time, all the solutions were maintained for 48 h at 20 ± 1 °C and 16 h light: 8 h dark in a climate chamber (PGC 1400, Bronson, Netherlands), and absorbance readings at 520 nm were made after 24 h and 48 h (Fig. S-1B and S-1C).

In our experimental conditions, the UV–Vis spectrophotometry method didn't have enough sensitivity to detect the 520 nm peak in the solutions with AuNP concentrations bellow 0.16 mg/l. Thus, only the solutions with concentrations in the range 0.16 mg/l to 20 mg/l were used to make a calibration curve (log absorbance versus log concentration). Positive and significant correlations (Pearson's correlation coefficient, here after indicated as r) were found for u.p. water ($N = 24$, $r = 0.996$, $p = 0.000$) and ASTM ($N = 24$, $r = 0.999$, $p = 0.000$).