The effect of silica-magnetite nanoparticles on the ecotoxicity of the antibiotic ciprofloxacin

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Abstract

Increasing production and application of engineered nanomaterials including nanoparticles (NPs) lead to their discharge into the environment, where they can interact with co-existing antibiotics from wastewaters, causing complicated joint effect to organisms that needs to be studied. Herein a typical engineered nanomaterial, silica-magnetite NPs modified with tetraethoxysilane and 3-aminopropyltriethoxysilane (MTA-NPs, 1-2 g/L), and a common antibiotic ciprofloxacin (CIP, 0–5 mg/L) were selected as the analytes. Their joint toxicity to a model ciliates infusoria Paramecium caudatum was specifically investigated. The impact of CIP, MTA-NPs and humic acids (HA) was tracked for 24 h individually and collectively on the survival of infusoria. The addition of MTA-NPs and HA at the studied concentrations lead to the survival of 60% of organisms. The combined presence of the MTA-NPs at a concentration of 1.5-2 mg/L and HA at a concentration of 20-45 mg/L has a multiplier effect and allows increasing the survival rate of ciliates > 70% due to the enhanced removal of CIP.

Introduction

Currently, the world has a problem of pollution of natural waters and soils with drugs, for example, antibiotics. (Gothwal and Shashidhar 2014). One such common antibiotic is ciprofloxacin (CIP). CIP is a widely used human, livestock and animal therapeutic agent (Davis et al. 1996). The CIP content in streams and wastewater has been found to be approximately <1 µg/L. However, the concentration of the antibiotic in wastewater from hospitals was significantly higher - 3-87 µg/l, and from enterprises for the production of drugs - 31 mg/l (Larsson et al. 2007).

Getting into aquatic natural objects, CIP affects the viability of living organisms to varying degrees. Thus, the concentrations of CIP at which various algal species lost 50% of their original population in the above toxicity studies ranged from 7.9 to 23,000 µg/L (Fu et al. 2017; Martins et al. 2012; Robinson et al. 2005). Eukaryotic microalgae are generally less sensitive than prokaryotes (Martins et al. 2012; Robinson et al. 2005). For Daphnia magna, 36.49 mg/l of the antibiotic leads to the death of 50% of the animals (Dionísio at el. 2019). Data on threshold concentrations of CIP for protozoa such as ciliates are not available. Therefore, one of the first tasks of this study was to study the ecotoxicity of CIP on ciliates.

The fate of CIP within aquatic systems is likely dependent on two most important mechanisms for its elimination from water: photodegradation (Cardoza et al. 2005) and sorption (Golet et al. 2002; Belden et al. 2007). When photodegraded, the half-life of CIP is approximately 2 hours in natural water, which probably leads to a loss of antibiotic activity (Cardoza et al. 2005). The presence of organic matter in the aquatic environment prolongs the half-life of CIP (Lin et al. 2017), which may result in antimicrobial activity on the solid matrix or on organisms that consume the solid matrix (Belden et al. 2007).

Dissolved organic matter (DOM) significantly affects the fate of antibiotics in the environment, being present in almost all aquatic ecosystems with a content of 0.1 to 10 mg/l. Humic matter (HM) is the most important part of organic matter in surface waters (Nebbioso et al. 2013). Essentially, humic substances
can be thought of as supramolecular structures composed of several thousand different molecules (Piccolo et al. 2001). To understand how antibiotics migrate in the environment, it is important to understand the mechanism of interaction between HS and antibiotics. This process is not well understood. Thus, the second task of this study was to evaluate CIP binding to humic acids (HA), representing naturally occurring HS in aquatic systems, prior to use nanoparticles of magnetite (MTA-NPs) and whether binding would affect the bioactivity towards test-organisms like ciliates. This was accomplished by measuring the sorption rate of CIP in a laboratory study with and without the presence of HS.

Like antibiotics, NPs are also a class of new contaminants that can accumulate and enter the environment. NPs are currently used in consumer products: cosmetics, therapeutics, drug delivery systems, food packaging, diagnostics, biosensors (Ray at el. 2009; Pereira et al. 2012; Pastrana-Martínez et al. 2015; Laurent et al. 2008). Due to increased use, NPs are being released into the environment more and more and are already a new class of pollutants of concern (Ray at el. 2009). Magnetite NPs undergo many chemical and physical changes when released into the environment, changing their surface. (Philippe et al. 2014; Aiken et al. 2011). It has been found that nearby chemicals from the environment readily coat the surfaces of the nanoparticles via surface ligand exchange. It has been found that nearby chemicals from the environment readily coat the surfaces of the nanoparticles via surface ligand exchange. It is assumed that, in an aqueous medium, Fe and O atoms on the surface of magnetite NPs will adsorb OH- and H+ ions. It is possible that due to the hydroxyl-rich surface, magnetite NPs will bind CIP at the carboxylic acid moiety (Ma et al. 2003; Rehana et al. 2015).

The use of NP magnetite and antibiotics continues to grow. This is likely to increase their environmental impact. Based on this, it is important to study not only the effect of individual pollutants, but also the collective effect.

The engineered magnetite NPs (MTA-NPs) are a prototype (model) of natural inorganic colloids mainly composed of iron oxides (Wigginton et al. 2007) and present in almost all surface waters and are part of aquatic ecosystems (Philippe et al. 2014). The high mobility and surface area of humic substances are known to play a key role in pollutant transport (Wigginton et al. 2007) and interact with microbial communities (Bonneville et al., 2006; Neal et al. 2005) and ciliates (Li et al., 2012). The behavior of colloidal nanoparticles depends on humic substances, as they are able to change their surface properties and hence their stability (Aiken et al. 2011), soil transport (Wang et al. 2012). In addition, HS strongly influence the adsorption of various ecotoxicants on colloids (Philippe and Schaumann 2014).

Therefore, this study was aimed to estimate joint effect of CIP and magnetite NPs in the presence of HA towards ciliates infusoria. Paramecium is a model organism that has made important contributions to molecular and cellular biology. Paramecium are visible to the naked eye due to their rather large size (50–300 µm in length) (Van Houten et al. 2019). R. Mayne et al. (2018) showed that P. caudatum cells consumed starch, which was coated with magnetite NPs in amounts exceeding 5-12% of their body volume. This proves that P. caudatum is a candidate organism for nanomaterial manipulation and
delivery. Magnetic restraints of Paramecium were also shown by S. Furukawa S and T. Kawano through internalized magnetite (particles about 3 µm in diameter) (Furukawa, et al. 2012). The surface modification silica-magnetite of NPs with silica (tetraethoxysilane and 3-aminopropyltriethoxysilane) presents a model of inorganic colloids.

**Materials And Methods**

**Chemicals**

CIP (P98%) was obtained from the Pharmaceutical factory "Kelun-Kazpharm" (Kazakhstan). Leonardite standard humic acid (POW-HA, Humintech) was used as received. The sample of silica-magnetite NPs modified with tetraethoxysilane and 3-aminopropyltriethoxysilane (MTA) has following characteristics: particle size ~12 nm, adsorption surface area - ~120 m²/g.

*Paramecium caudatum. Acute Toxicity Test*

The acute toxicity test based on ciliates survival was conducted on *Paramecium caudatum* Ehrenberg as described previously (Bondarenko et al. 2020). Briefly, this test measures the mortality of *Paramecium caudatum* when exposed to a toxicant, compared to control. The assay was performed in 96-well polystyrene plates (well size 1 mL, Eppendorf). Stock cultures of *P. caudatum* were maintained in the mineral Lozin-Lozinskiy nutrient medium with the following composition, mg L⁻¹: NaCl—100.0, KCl—10.0, CaCl₂·2H₂O—10.0, MgCl₂·6H₂O—10.0, NaHCO₃—20.0 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Stock cultures were maintained at room temperature (22 ± 2 °C), pH 7.5–8.0, and a yeast suspension was added as feed. To start the test culture, about 1/3 of the stock culture was transferred to a Petri dish with fresh nutrient medium and incubated for 24 h at 22 ± 2 °C in the dark.

Using a stereoscopic microscope (Model MC-1, Micromed, Shanghai, China), 10–15 ciliates were transferred with a capillary pipette into each of 3–4 test wells containing fresh culture medium. The volume of liquid used to transfer the ciliates into the wells did not exceed 0.02 mL. In general, each series of wells (control and test wells) contained at least 30 ciliates. To the control wells, 0.6 mL of culture medium was added, and to the test wells, 0.6 mL of test sample was added. The plates with the samples and ciliates were incubated in the dark at 22 ± 2 °C. No food or other supplements were added during the exposure period. After 24 h of incubation, the viability of individuals in each well was checked under a stereomicroscope. Freely moving ciliates were considered to be alive, and immobile individuals were considered to be dead. The mean values were calculated and compared with the values of the control. The results were considered reliable if the mortality of the control did not exceed 10%.

**Bacterial Acute Toxicity Test**

Bacterial Acute Toxicity Test was conducted as described previously (Yakimenko et al. 2022). Bacterial toxicity was determined by a bioluminescence inhibition test as described by (Zarubina et al. 2015). We used lyophilized bacteria with a genetically modified strain of luminous Escherichia coli K12 TG1 carrying
the lux operon of luminous soil bacteria *Photorhabdus luminescens* ZM1. A bioluminescent strain *E. coli* K-12 TG1 hsdR17 hsdM thi relA1 supE44 Δ(lac-proAB) F′(traD36 proAB+ lacIq lacΔZM15) (pXen7) was obtained by transformation with a multicopy hybrid plasmid vector pUC18 with an introduced EcoRI DNA fragment from *Ph. luminescens* ZM1 with the size of approximately 7 kb. This genetically modified strain of *E. coli* K12 TG1 encodes the entire lux operon of *Ph. luminescens* ZM1 with luxCDABE structural genes (Manukhov et al. 2000). The strain was produced and stored in the collection of the Microbiology Department of the Faculty of Biology, Moscow State University (Danilov et al. 2002).

The lyophilized bacterial cells were used after rehydration for 30 min in 10 mL of cooled sterile distilled water (pH 7.4), and a suspension of 2.3–2.7×10⁷ cells ml⁻¹ was used. The density of the bacterial suspension was measured using a photoelectric colorimeter (λ = 670 nm) and expressed as the number of cells in ml according to the calibration curve constructed beforehand.

Bacterial suspension (0.1 ml) and 0.9 ml of PE test solutions (or distilled water as control) were placed into vials and the luminescence intensity (I) was measured after 30 min of exposure using a Biotoks-6MS luminometer (Russia) recording it in counts per second.

The sample toxicity was assessed using toxicity indicator (T):

\[ T = \left( I_0 - \frac{I}{I_0} \right) \times 100 \]

where \( I_0 \) and \( I \) are luminescence intensities of the control and experimental samples in counts per second, respectively, at the fixed exposure time (30 min) at room temperature (22°C). \( T \) was determined automatically using the in-built software of the luminometer.

The toxicity was classified using the generally recognized approach: \( T < 20 \) permissible toxicity; \( 20 \leq T < 50 \) toxic; \( T \geq 50 \) highly toxic. If \( T < 0 \) (a sample stimulates bacterial luminescence), the sample is assumed to be nontoxic (Danilov et al. 2002; Zarubina et al. 2015).

**Equilibrium sorption studies**

A sorption technique was used to quantify CIP and MTA-NPs sorption in the presence of HA. 1 and 2 g/L of MTA-NPs and/or HA 0.01 and 0.05 g/L was weighed in and placed into a 200 mL conical flask and filled with 100 mL of distilled water with CIP concentration of 5 mg/L to conduct the adsorption experiments. All flasks were rotated at 30 rpm in the dark on a rotary shaker for 24 h at 298 K. After the reaction time of 24 h, the MTA-NPs and the supernatant were separated as described above. At the end of the equilibration period, the suspensions were centrifuged at 1000 rpm for 10 min and the supernatants were extracted by syringe and filtered through a hydrophilic membrane (0.45 lm) for CIP quantification by the UV-Vis spectrophotometry. After filtration, the pH of the supernatant was measured. Its values are given in Table 1. Equilibrium CIP concentrations were determined by transferring 2 mL aliquots to a quartz cuvette. Samples were analyzed on a Solar PB2201 UV-Vis spectrophotometer in cuvettes.

**Design of experiment**
Response Surface Methodology (RSM) is a combination of statistical and mathematical approaches to identify the best conditions for conducting an experiment. Design-Expert 13 software was used to generate statistical models. The surface response methodology was used to evaluate the relationship between a number of independent factors and responses in order to optimize the latter.

The inhibition of survival of infusoria *Paramecium caudatum* (% to the control) is the response of the system (Y) and the two parameters including MTA-NPs and HA concentrations with three levels [low (-1), high (+1) and median (0)] were independent variables. The typical concentration of HA ranges from 1 to 50 mg L\(^{-1}\) (carbon) in soils and groundwater (Wang et al. 2012). The ranges of factors and levels of independent variables are shown in Table 2.

**Statistical analyses**

Comparisons among EC50 values for different C(HS) and different pHs were analyzed using two-factor analysis of variance (ANOVA; C(HS) as factor one and pH as factor two). Comparison among treatments was conducted using Fisher’s probable least-squares difference test (PLSD; \(p < 0.05\)). Individual comparisons between pH levels for each C(HS) were also performed (t-test) to enhance the description of a positive interaction noted for the two-factor ANOVA. All computations were made using Statview (SAS Institute, Version 5.01, Cary, NC, USA).

**Results And Discussion**

**Bactericidal action of ciprofloxacin**

CIP is a bactericidal drug, affecting gram-negative and gram-positive organisms (Yakovlev 1997). Therefore, first of all, it is important to trace how much this antibiotic affects the persistence and activity of environmental bacteria.

Environmental bacteria are ubiquitous, incredibly diverse, and play a crucial role in the cycling of elements within our environment (Knoll et al. 2012). No effect of CIP on luminous bacteria was observed at CIP concentrations of 10.20 mg/L. The toxicity index was 0 (Fig. 1). The luminescence of the biosensor increased after the addition of predetermined concentrations of the antibiotic. When the concentration of CIP was increased to 100 mg/L, the toxicity index reached a value of 29.11, which indicates a toxic effect. The degree of toxicity of CIP was osseous at 150 mg/L.

The calculation of effective concentrations using a probit regression model showed that the EC50 for the bacterial test-culture is 135.73 mg/L of CIP, and the EC10 is 66.7 mg/L.

**Toxicity of CIP, MTA-NPs and HA on infusoria**

The CIP evaluated for the current study’s toxic effects were toxic to ciliates - infusoria *P. caudatum* in the survival inhibition test: the EC50 value was reached at 1.1 mg/L. The mortality of ciliates proportionally increased with increasing antibiotic concentration, reaching a peak at 5 mg/l CIP (inhibition of survival ~
80%). A dose of 0.05 mg/L CIP resulted in inhibition of survival in less than 20% of ciliates and can be
called not harmful (fig. 2).

Comparison of the toxicity of CIP in relation to ciliates and bacteria showed that ciliates were much more
sensitive to CIP than bacteria. This justifies the choice of ciliates as test objects in detoxification
experiments.

The impact of CIP, MTA-NPs and HA was tracked for 24 h individually and collectively on the survival of
infusoria. The addition of MTA-NPs and HA at the studied concentrations did not lead to the death of
more than 40% of living organisms (Fig. 3) (p-value for different concentrations less than 0.05). According
to numerous published data, iron oxide NPs functionalized with amine-containing silanes exhibit different responses with respect to various test organisms. The mechanism of toxicity of
magnetite NPs with APTES can be described with the function of the positive charge of the \(\text{NH}_3^+\) ions,
which presumably interacts with negative charges on the surface of microorganism cells (phospholipids),
leading to a change in membrane permeability and leakage of intracellular components (Bieser and Tiller
2011; Fernandes et al. 2013). Similar effects were also observed by the authors (Hoskins et al. 2012) for
iron oxide NPs coated with polyethyleneimine (PEI) in comparison with the same NPs additionally coated
with polyethylene glycol (PEG), which affect the negatively charged cell membrane and enhance
endocytosis. Primary amines on the surface of NPs create a large positive surface charge (+55.6 mV)
and, as previously reported, cause a cytotoxic effect (Fernandes et al. 2014).

The combined presence of MTA-NPs and dissolved organic matter in a solution leads to inhibition of the
survival of ciliates by less than 20%, which is lower than for a single MTA and HA (Fig.3).

This made it possible to choose the following concentrations for a multifactorial experiment to assess
the detoxifying ability of silica-coated magnetically controlled MTA-NPs in the presence of DOM. Further
work was devoted to the evaluation of the effect of different concentrations of MTA-NPs in combination
with different concentrations of HA on the detoxification of CIP using the Design of experiments (The
response surface methodology, RSM).

**Regression models and statistical testing**

The results of mathematical and statistical processing using the surface response methodology made it
possible to obtain a second-order equation showing the dependence of the inhibition of the survival of
ciliates on the concentration of nanoparticles and dissolved organic matter:

\[
Y \text{ (Inhibition of survival)} = 32.29 - (6.40A) - \\
-(8.89B) - (4.22A^2) + (22.94B^2) + (10.01AB)
\]  

(1)

where A and B refer to the concentrations of MTA-NP and HA, respectively. Equation (1) shows how the
inhibition of survival of ciliates changes when one of certain factors or their combination changes. The
sign in front of the coefficient of the regression equation indicates the nature of the change in the
response. A positive value means that the response - the survival of ciliates - increases with an increase in
this factor, a negative value - vice versa. In this case, both factors are negative. Thus, a decrease in the concentration of nanoparticles and dissolved organic matter leads to an increase in the mortality of ciliates. The value of the coefficient of the regression equation makes it possible to estimate which change in which of the factors leads to the most significant change in the response. So, according to the data obtained, it is the change in the concentration of humic substances that most affects the survival of ciliates.

**Validity of the model**

The adequacy of the model describing the effect of nanoparticle and DOM concentrations on ciliate survival was assessed using analysis of variance. Multiple regression analysis made it possible to obtain coefficients adequately described by a quadratic model. The results of the analysis of variance for quadratic response surface models are presented in Table 3.

The value of the coefficient of determination ($R^2$) equal to 0.86 indicates that more than 86% of the total data can be explained by a quadratic model and less than 14% cannot be described by the resulting regression analysis model. The adjusted regression values for the data obtained were greater than 0.83, indicating sufficient correlation between the response and the influencing factor. The Predicted $R^2$ of 0.8046 is in reasonable agreement with the Adjusted $R^2$ of 0.8376; i.e. the difference is less than 0.2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination. In this particular case the value is well above 4.

The p value for the model is less than 0.05, which indicates that the model is significant, and therefore has a significant effect on the response under study (infusoria survival inhibition). In the same manner, for the inhibition of survival all factors, their squares and linear combination ($A, B, B^2, AB$) except for the value of the squared factor $A^2$ (MTA-NPs concentration) are significant model terms. These insignificant model terms (not counting those required to support hierarchy) can be removed and may result in an improved model. According to the F-criterion of the model equal to 36.06, the model can be called significant. With a probability of 99.99%, such a large value of the F-criterion cannot arise due to noise.

The constructed graphs of the predicted and actual and normal probabilities of student residuals make it possible to assess the suitability of the developed statistical model. The relationship between the experimental and predicted values of survival inhibition in the presence of MTA-NP and DOM is shown in Fig. 4.

According to Figure 4a, there is a good correlation between the data obtained experimentally and the values predicted by the statistical model for the ciliate survival inhibition values. The normal probability plot can be used as a method for graphical evaluation of the normality of residuals, that is, the difference between experimental and model data (Fig. 4b). From fig. 4b shows that the residual behavior follows a normal distribution and is linear, which indicates the adequacy of the model: this model can be used to assess the detoxification of MTA in the presence of DOM.
Effect of variables on responses

Effect of the concentrations of MTA-NPs on the detoxication process

Figure 5a represent the dependence of the value of inhibition of ciliates at various concentrations of the MTA-NPs from 0-2 g/l in the absence of DOM (HA concentration equals 0 g/L).

The absence of the MTA-NPs leads to a death of 79.1% on average due to the toxic effect of 5 g/L CIP according to experimental data, which is in good agreement with the model values of 76.3% and literature data (Dionísio et al. 2019). The addition of the MTA-NPs leads to an almost linear decrease in the inhibition of infusorias from 79.1% to 44.6% with an increase in the MTA-NPs concentration from 0 to 2 g/L, which indicates the sorting of CIP on the MTA surface and a decrease in the concentration of CIP in the solution after removal of the precipitate. Sorption of CIP is also confirmed by UV spectrophotometry data demonstrating the removal of about 50% of CIP with the addition of 1 g/L MTA-NPs.

The mechanism of sorption can be explained by the forces of electrostatic attraction between MTA-NPs and CIP. For the pKa of CIP, the carbonyl group get deprotonated at 5.9 (pKa1) while the amine group gets protonated at 8.9 (pKa2) (Balarak et al. 2016; Bizi et al. 2020). CIP can thus exist as a cation, anion and zwitterion (Carabineiro et al. 2011). The position of functional group of MTA-NPs and CIP can be observed in Figure 6 in green and red. At the pH close to 7, the NPs with negative surface charge due to the presence NH2-groups on the surface (zeta potential of about -25 mV at pH about 7) have a complex interaction by deprotonated carboxylic groups of CIP in the pKa interval 5.9–8.8 (pKa1 = 5.90±0.15, pKa2= 8.89±0.11 (Carabineiro et al. 2012) (Fig 6).

Previously, Rakshit and colleagues showed an electrostatic pH-dependent sorption mechanism for ciprofloxacin (Rakshit et al. 2013). CIP (pKa1 = 6.1, pKa2 = 8.7) and magnetite (approx. PZNPC is 6.5) are positively charged at acidic pH, which limits their interaction. The increase in pH from 4.0 to 6.0 leads to an increase in the proportion of neutral CIP and to the possibility of interaction between CIP and magnetite. Adsorption decreased at pH above 6.5, because. CIP and magnetite became increasingly negatively charged with increasing pH.

The EC50 value during detoxification of CIP in the presence of MTA-NPs is 1.7 g/L. The resulting regression equation (1) indicates that an increase in the concentration of NPs will lead to a decrease in the inhibition of the survival of protozoa: the addition of MTA-NPs at a concentration of 3.2 g/l will lead to the complete survival of ciliates.

Effect of DOM concentration on the detoxication process

Figure 5b demonstrates the dependence of the inhibition of survival of ciliates on the DOM concentration in the absence of MTA-NPs (0 g/L). The graph shows that the dependence is non-linear: the inhibition of survival decreases with an increase in the concentration of HA from 0 g/L (mortality of 63% of ciliates) to 0.029 g/L (mortality of 31% of ciliates). An increase in the concentration of HA leads to an increase in the
inhibition of the survival of ciliates up to 46% at the maximum concentration of HA used 0.05 g/l. The predicted data shows, that a further increase in the concentration of HA to 0.07 g/L will lead to the death of 100% of ciliates.

Our earlier assessments of the toxicological ability of HA (Bondarenko et al. 2020) showed that they did not show their own toxicity to ciliates in the concentration range from 0.1 to 100 mg/L. This allows us to conclude that an increase in the inhibition of test organisms with an increase in the concentration of HA is not associated with the intrinsic toxicity of the dissolved organic matter, but is related to sorption issues. UV spectrophotometry data show that an increase in the concentration of HA from 0.01 g/L to 0.05 g/L leads to sorption from 90 to 60% of CIP.

The non-linear dependence of the concentration of adsorbed CIP and the sequence of survival of ciliates on the concentration of HA may be associated as with steric hindrance during sorption as well with more difficult processes.

The ability to dissolve in alkaline and neutral solutions depends on the chemical composition of HA (Stevenson 1994). Carboxyl and phenolic groups of HA in an alkaline medium are deprotonated, negatively charged groups are repelled, because of this, HA molecules acquire an elongated shape. With a decrease in pH, phenolic and carboxyl groups are protonated, the repulsive effect decreases, because of this, the molecules acquire a helical conformation. In this case, the hydrophobic areas are located in the inner part of the structure, and the hydrophilic parts are in contact with the aqueous medium. At the same time, an increase in the concentration of HA can lead to steric hindrances in the sorption of CIP due to the inaccessibility of reactive groups. Fang et al., 2013 (Fang et al. 2013) suggested that the HA molecule could act as an electron shuttle during the reaction, promoting CIP degradation due to accelerated electron transfer. For example, the decomposition of CIP occurred predominantly at a lower concentration of dissolved HA. This increased the removal of CIP from the solution. At high HA concentrations, HA reactive centers were depleted and CIP removal dropped to 0.

**Effect of joint presence of MTA-NPs and DOM on the detoxication process**

The presence of HA as a dissolved organic matter during the sorption of CIP by MTA-NPs has a complex effect. Figures 7a and 7b show 3D and 2D graphs of the inhibition of the survival of ciliates in the presence of HA and MTA-NPs of various concentrations. According to the concentration of HA, there are three regions that differ in the survival of ciliates: at a concentration of HA up to 20 mg/l and over 45 mg/l, regardless of the concentration of the MTA-NPs, the survival of ciliates is suppressed by 40% or more; in the range from 20 to 45 mg/l of HA, the inhibition is also controlled by the concentration of the MTA-NPs and reaches 30% or less. As can be seen, the highest survival rate of ciliates (more than 70%) is observed at a concentration of HA from 10 to 45 mg/l and at a concentration of MTA-NPs from 1.25 to 2 mg/l. The presence of HA at a concentration of less than 20 mg/L, regardless of the concentration of the MTA-NPs, leads to the mortality of 40% or more of ciliates, which correlates with data on detoxification in the absence of HA (Fig. 5a) or in the absence of MTA-NPs (Fig.5b). This indicates the absence of
multiplicative sorption of CIP with the simultaneous presence of HA and NPs: it can be assumed that CIP competes with HA adsorbed on the MTA surface and occupying their reactive centers.

On the other hand, the presence of HA at a concentration above 45 mg/L also leads to the death of more than 40% of ciliates, regardless of the concentration of the MTA-NPs, which confirms the key role of HA in the detoxification of CIP (which is also indicated by the values of the regression coefficients in equation 1). The absence of any effect of NPs on the survival of ciliates at HA concentrations above 45 mg/L also indicates the likelihood of adsorption of HA on the surface of MTA-NPs with overlapping of its reactive centers. Singh et al (Singh et al. 2009) also studying the sorption of radionuclides on the surface of magnetite in the presence of HA, did not see a significant change in the sorption capacity at a concentration of HA from 2 to 20 mg/L. The only explanation can be that even at the highest HA concentration (100 µ mol/L), HA may be completely sorbed by magnetite at all pH values as was also observed by (Illes and Tombacz 2004). Despite that HA have a negative charge in the entire range of pH (Tombacz at el. 2015), and NPs is negative in the study area (zeta potential of about -25 mV at pH about 7), it is most likely to assume that the MTA-NPs surface is completely covered with HA at their concentration above 45 mg/L. As a result, the magnetically active NPs in the presence of HA adsorbed on its surface behaves in accordance with the nature of the surface, which is consistent with (Saei et al. 2017), who stated that it is surface characteristics that stands out as one of the most significant determinants of biological performance, as the NP surface is the most prominent and earliest point of exposure (Saei et al. 2017).

However, the combined presence of the MTA at a concentration of 1.5-2 mg/L and HA at concentrations of 20-45 mg/L has a multiplier effect and allows increasing the survival rate of ciliates to 70% or more due to the removal of a higher concentration of CIP. Luo et al. (2019) also demonstrated a decrease in the sorption capacity of biochar in relation to CIP in the presence of high concentrations of HA. However, according to the presented data, the sorption of CIP on biochar in the presence of HA decreases from 66.7% at 5 mg/L of HA to 0% at 20 mg/l and remains the same with an increase in the concentration of HA to 50 mg/l. Luo and colleagues explained this by the fact that a large amount of HA in solution is adsorbed on biochar, which can lead to blockage of pores or competition with CIP for sorption sites. On the other hand, as previously described, HA can act as an electron shuttle (Fang et al. 2013) and lead to the decay of the CIP molecule during the electron transfer reaction.

Conclusions

One of the important ecological results of the work was that the combination of MTA-NPs and HA in solution prevented the death of ciliates from the presence of CIP, but separately these substances acted differently on ciliates. MTA-NPs are often considered harmless, but the results show the potential activity of these particles in aqueous solution. The addition of MTA-NPs to a solution with CIP (5 mg/L, the mortality rate of the ciliates of more than 80%) in a concentration of 3.2 g/L leads to a complete survival of the ciliates. Adding HA to a solution with CIP (5 mg/L, mortality of ciliates of more than 80%) first leads to a decrease in the mortality of ciliates at the concentration of HS 29 mg/L, and then to an
increase in the mortality of the ciliates, reaching a maximum (100%) at 70 mg/L HS. The highest survival rate of the ciliates (more than 70%) is observed with the concentration of HA from 10 to 45 mg/L and the concentration of MTA-NPs from 1.25 to 2 mg/L.

The infusoria _P. caudatum_ were more sensitive to pollution of the aquatic environment with CIP than other groups of organisms, because EC50 for infusoria amounted to 1.1 mg/L. This is much less than for the soil bacteria _Photorhabdus luminescens_ ZM1 EC50 (135 mg/L).

Thus, an important result was obtained from an environmental point of view. Understanding the interaction of humic acids, nanoparticles, and antibiotics may be critical to fully elucidate the mechanism of transport of both colloidal nanoparticles and pollutants in the environment.

**Declarations**

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**References**


Tables

Tables 1 to 3 are available in Supplementary Files section.

Figures

Figure 1

Change in the toxicity index (T) of CIP in relation to bacteria

Figure 2

The number of dead ciliates in the aquatic environment with different concentrations of CIP

Figure 3

Inhibition of survival in the test with ciliates Paramecium caudatum of CIP, MTA-NPs, HA and MTA-NPs-HA in 24 h immobilization at pH ~ 7. All concentrations are nominal. HA–humic acids, MTA – Fe3O4-TEOS-APTES NPs, CIP- CIP. Average values ± SD of triplicate
Figure 4

Actual and predicted values of response for evaluation of infusoria’s (a) and normal probability plot (b) of the studentised residuals
Figure 5

(a) Influence of MTA-NPs concentration on ciliates survival in experiments on CIP detoxification (HA concentration = 0 g/L). (b) Influence of the concentration of dissolved organic matter on the survival of ciliates in experiments on the detoxification of CIP (the concentration of the MTA-NPs = 0 g/L). (red dots - experimental data, dotted line - 95% confidence interval)

Figure 6

Probable mechanism of interaction between MTA-NPs and CIP at different pH values
Figure 7

3D (a) and 2D (b) graphs of the inhibition of the survival of ciliates in the presence of HA and MTA-NPs of various concentrations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Table3.pdf