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ORIGINAL PAPER

Transformation of microbiota of fish intestines and gills against the background of molybdenum oxide nanoparticles in environment

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Abstract
This work is aimed at the evaluation of transformation of microbiota of *Danio rerio* intestines and gills under the impact of MoO_3NPs, which corresponds to the existing level of studies using this model. The composition of the microbial community of *Danio rerio* was studied after nanoparticles of MoO_3 (MoO_3NPs) were administered into the environment in amount of 0.2 mg/dm^3 within 7 days (Group II), in amount of 0.4 mg/dm^3 within 14 days (Group III) in the form of lyosols with fish feed. MoO_3NPs were not added to the reference group I; the procedure was comprised of the following: sampling, isolation, cleaning, measurement of DNA concentrations, polymerase chain reaction, validation and normalization of libraries with subsequent sequencing on the basis of high-performance sequenator (MiSeq Illumina, USA). Dose-dependent influence of MoO_3NPs on microbiota transformation of intestines and gills has been estimated. Nanoparticles modify the composition of the microbiota by reducing the amount of symbionts participating in vital activity of macro-organism. Gills' microbiota is modified to a greater extent, and the increased occurrence of Actinobacteria phylum has been detected with significant difference in Group III. In fish intestines of Group II *Cetobacterium somerae* is the significant species. In Group III, representatives of *Acinetobacter* and *Staphylococcus* genuses have been identified, and the increase in fraction of Gram-positive microflora has been observed. The results evidence the violation of equilibrium in microbiota of intestines and gills and suppression of protecting mechanisms which usually prevent colonization by foreign microflora. Penetration of MoO_3NPs into organism can influence the fish intestinal and respiratory systems and health.

Keywords Microbiota · Intestines · Gills · *Danio rerio* · Metagenomic sequencing · Molybdenum nanoparticles

Introduction

Development of nanotechnologies is accompanied by increasing production of nanoparticles (NPs) and expansion of scope of their application, which leads to their controlled and non-controlled penetration into environmental systems and, as a consequence, into living organisms and occurrence of previously unknown risks. The new risks are determined by the existence of unique physicochemical properties of nanomaterials due to their small sizes, significant specific surface area and higher reactivity in comparison with substances in macro-phases (Shaw and Handy 2011; Osborne et al. 2013; Wang et al. 2014; Miroshnikova et al. 2015). The increasing production and wider application scope can be exemplified by NPs containing molybdenum (Naylor et al. 2016; Tadi et al. 2016). NPs of molybdenum and its compounds are characterized by unique biological properties (Sam et al. 2015; Qureshi et al. 2016), determining a wide range of effects on environmental systems (Kosyan et al. 2015; Lebedev et al. 2016). They are widely applied in modern technologies (Naylor et al. 2016; Chen et al. 2016) including multifunctional electric catalysis (Tadi et al. 2016) and production of lubricants (Parenago et al. 2002). In addition, ultrafine products of molybdenum and its compounds are characterized by unique biological properties and can be applied for therapy of tumors (Liu et al. 2015), as

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antimicrobial (Fakhri and Nejad 2016; Zhang et al. 2016) and fungicide substances (Qureshi et al. 2016), for growth stimulation of blue–green algae (Sam et al. 2015).

As a result of this, an uncontrolled release of nanoparticles into the environment and living organisms occurs and it implies the study of their danger/safety of living organisms. Meanwhile, the information on consequences of interaction between the produced NPs and biological entities is insufficient.

In this regard, certain interest is felt in the indirect influence of molybdenum NPs on microbiota of animals. The existence of such influence was several times confirmed for nanomaterials (He et al. 2013; Yausheva et al. 2016). Variations in microbiocenosis of intestines under the action of NPs are negative, leading to decreased growth, high susceptibility of fishes to diseases and increased fatality. Stresses of various origins inevitably upon intensive cultivation worsen the situation.

The use of *Danio rerio* as a biological model for the study of biological effects of metal nanoparticles is reasonable and corresponds to the current level of research. In particular, the study of the microbiocenosis of these fish and its changes after exposure to nanoparticles of various metals found a sufficient response in the studies of various authors. Thus, the microbiocenosis of *Danio rerio* fish was studied under the influence of copper nanoparticles (Griffitt et al. 2007), silver (Asharani et al. 2008; Osborne et al. 2015; Devi et al. 2015), titanium oxide (Clementea et al. 2014) and other metal nanoparticles (Kovriznych et al. 2013). We have shown the transformation of microbiocenosis of gills and intestines of *Danio rerio* in response to the presence of molybdenum oxide nanoparticles in the feed for the first time.

Materials and methods

Experimental animals and housing conditions

Microbiota of gills and intestines were studied using *Danio rerio* specimens (freshwater fish belonging to the minnow family), 1 month old, of equal weight and gender, and without any signs of diseases. The fishes were kept in aquariums made of silicate glass with 10 L in capacity and equipped with filtration system and water saturation with air oxygen. The fishes were fed once per 2 days with fish feed (*Chironomidae* frozen larvae). Conditions of growing and keeping of the considered specimens met the requirements of Organization of Economic Cooperation (OECD 1992). All of the experimental methods and techniques were approved by the Committee on Ethics of the Federal Research Centre of Biological Systems and Agro-technologies.

In order to perform experiments by paired comparison method, three groups of *Danio rerio* were arranged ($n = 10$): reference group I (control)—without the addition of MoO_3NPs—and two experimental groups II and III—to these aquariums the considered MoO_3NPs were added in amount of 0.2 mg/dm^3 in the form of lyosols with fish feed once per seven days (Piccinetti et al. 2014). In Group II, the exposure time was 7 days and in Group III—14 days. The experiment was repeated three times.

Characterization of nanoparticles

The considered MoO_3NPs were obtained by plasma chemical synthesis (OOO Platina, Moscow). The MoO_3NPs were certified by scanning electron microscope (JSM 7401F) and transmission microscope (JEM-2000FX, JEOL, Japan), by X-ray phase analysis using a DRON-7 multiphase diffractometer (NPP Burevestnik, Russia) (Fig. 1a, b); the determined physicochemical properties were as follows: 69.8% Mo and 30.2% O_2; particle size 92 nm; specific surface area 12 m^2/g; and Z-potential -43 ± 0.52 mV. In order to prepare lyosols, the MoO_3NPs were dispersed (UZDN-2T, NPP Akadempribor, Russia, f-35 kHz, 300 W, A-10 μA, 30 min).

DNA isolation and microbiota analysis of gills and intestines

Microbial biodiversity of *Danio rerio* gills and intestines was estimated on the 7th and the 14th days; the procedure was comprised of the following: sampling, isolation, cleaning, measurement of DNA concentrations, polymerase chain reaction, validation and normalization of libraries with subsequent sequencing on the basis of high-performance sequenator of second generation (MiSeq Illumina, USA). The obtained results were analyzed using the data by Cantas et al. (2012) and Ringo et al. (2016). Samples of gills and intestines were extracted from fish bodies by sterile disposable pincer and placed into sterile Eppendorf microtubes (Nuova Aptaca SRL, Italy). Then the gill mucus and content of gastrointestinal tract were used for isolation of purified DNA preparations according to the modified procedure (Andronov et al. 2011). After isolation and purification, the DNA concentration was measured in solution using two methods: by NanoDrop instrument (Thermo Scientific, USA) in order to plot cures of DNA optical density and estimation of OD (260)/OD (280) and by Qubit 2.0 instrument (Invitrogen/Life Technologies, USA) in order to determine concentration in ng/μl. DNA concentrations were measured three times: after DNA isolation, after the first polymerase chain reaction with specific 16S prokaryotic primers and after the second polymerase chain reaction with adapters and indices of Nextera XT protocols. Further analysis of microflora was based on metagenomic sequencing.

The reads (R1 and R2) were combined by means of PEAR software (paired-end assembler, PEAR version 0.9.8, April

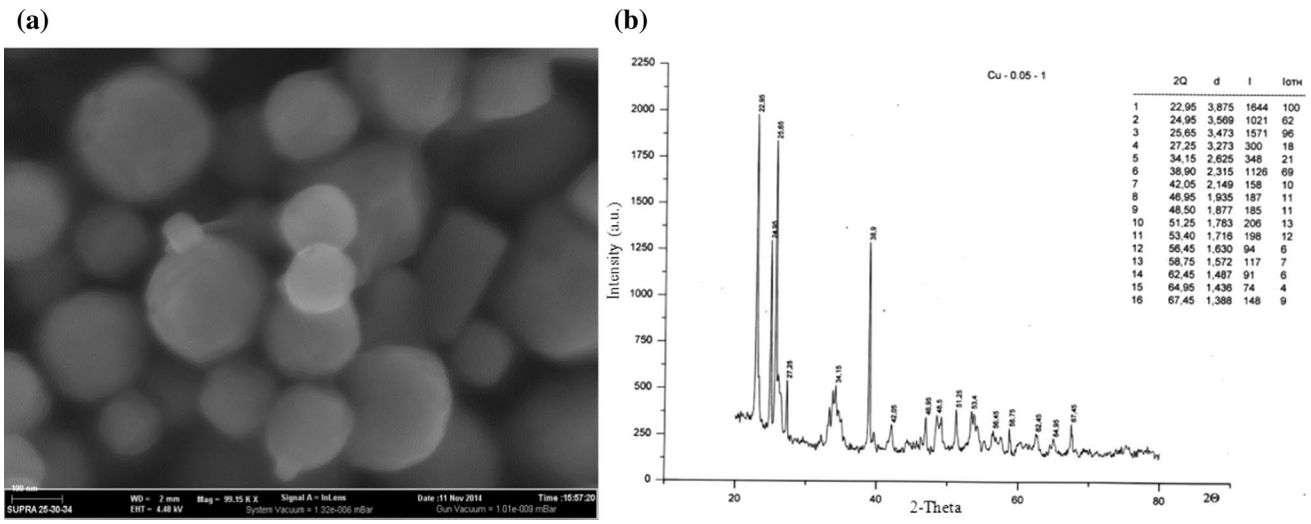

Fig. 1 Scanning electron microscopy MoO_3NPs (**a**); X-ray diffraction pattern MoO_3NPs (**b**)

9, 2015) with the following parameters: minimum overlap—40 bp, *P* value—0.001, quality $Q = 30$ (http://www.exelixis-lab.org/web/software/pear) (Zhang et al. 2014). Filtration, dereplication, elimination of chimeric sequences, clustering, sorting (rejection of singletons) and elimination of contamination were conducted using USEARCH software (USEARCH version 8.0.1623_i86linux32 (C) Copyright 2013-15 Robert C. Edgar, all rights reserved). Filtration was based on -fastq_filter algorithm with the parameters: minlen 415 bp (minimum sequence length: 415 nucleotides) and truncqual 15 (minimum quality of reading: $Q = 15$). Replication was based on -derep_prefix algorithm. Clustering and elimination of chimeric sequences were based on -cluster_otus algorithm (http://drive5.com/usearch) (Edgar 2010). Visualization was based on the visualization and analysis of microbial population structures (VAMPS https://vamps.mbl.edu/) (Huse et al. 2014).

Statistical analysis was performed by comparison of experimental groups with reference group using SPSS 19.0 (IBM Corporation) and Statistica 10. The value with $P \leq 0.05$ was considered to be statistically significant. To create an illustration, we used MS Office Excel.

Results and discussion

Results

The study of intestinal microbiocenosis *Danio rerio*

Using 16s rRNA as marker, it was revealed in microbiota of *Danio rerio* intestines that the dominating taxon was *bacteria*; herewith, its occurrence was about $99.6 \pm 2.51\%$ of total analyzed specimen in reference group I (Fig. 2). Herewith, 18 phyla were classified, *Proteobacteria* were isolated as dominating ones ($93.01 \pm 2.32\%$ of total amount), and the following were also included: *Firmicutes*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Cyanobacteria* and *Bacteroidetes*; however, their content was 2.49 ± 0.021, 1.0 ± 0.021, 0.89 ± 0.017, 0.56 ± 0.008, 0.3 ± 0.005 and $0.3 \pm 0.005\%$ of total amount, respectively, which was not higher than 3.5%.

Taxonomic diversity of *Proteobacteria* phylum was comprised of three classes: *Gammaproteobacteria*, *Betaproteobacteria* and *Alphaproteobacteria*. Other classes, not exceeding 3.5%, were *Firmicutes* taxon (2 classes), *Actinobacteria* (1 class) and *Planctomycetes* (1 class). Total content of non-classified classes was 1.53%. Dominating position among families of microbiota of intestines was occupied by *Enterobacteriaceae*, *Aeromonadaceae* and *Pseudomonadaceae*; all they were referred to *Gammaproteobacteria* class.

In turn, *Enterobacteriaceae* were presented by the following genuses: *Citrobacter*, *Enterobacter* and *Plesiomonas*. Minor taxa (below 3.5%) were presented by *Shewanella* and *Shinella*.

Microbiota diversity of *Danio rerio* intestines was presented by 269 morphologically different bacterial species, among which only four were significant: *Enterobacter soli*, *Citrobacter freundii*, *Tolumonasauensis* and *Citrobacter werkmanii*, herewith, non-classified types dominated.

Study of microbiota of *Danio rerio* intestines in Groups II (Fig. 3) and III (Fig. 4) revealed variations of both the number of previously determined types and taxonomic composition. Thus, upon single addition of MoO_3NPs, *bacteria* taxon remained to be dominating ($99.3 \pm 1.86\%$ of total number), though 16 phyla were classified, among which *Proteobacteria* also dominated but with the decrease in number

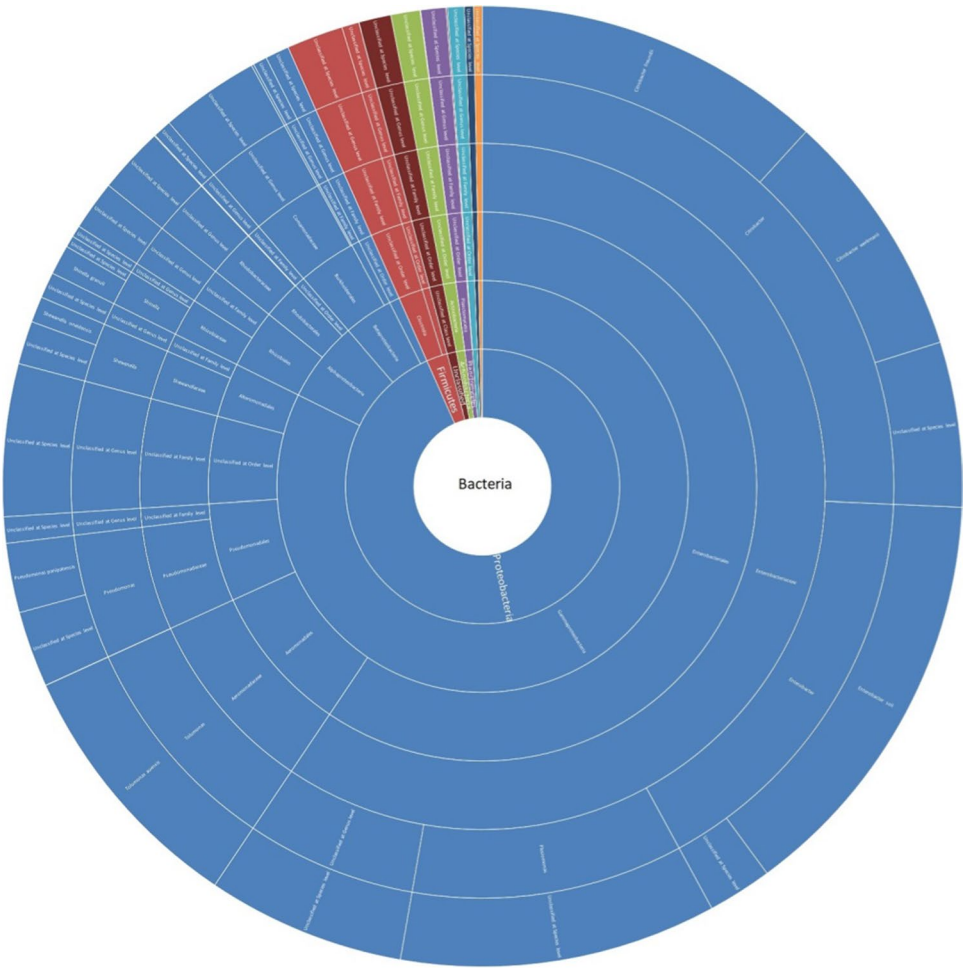

Fig. 2 Taxonomic composition of intestinal microbiota *Danio rerio* of the control group I

by 21.35 ± 0.58% in comparison with reference group. Taxa with the number in excess of 3.5% also included *Actinobacteria* and *Fusobacteria*, not identified in reference group.

Repeated addition of MoO_3NPs (Group III) did not vary the number of bacterial representatives. Thus, in Group III the number of *bacteria* was 99.5 ± 1.63% of total; however, the number of *Proteobacteria* decreased by 32.68 ± 0.89% in comparison with reference group and by 11.3 ± 0.28% in comparison with Group II. At the same time, the number of *Fusobacteria* phylum tended downward and was only 1.12 ± 0.02%, which was identical to its content in reference group.

In Group II, *Proteobacteria* taxon was presented mostly by *Alphaproteobacteria* class occupying more than one-half of total number of these taxa; *Gammaproteobacteria*, *Actinobacteria* and *Fusobacteria* classes were less in number. Herewith, 30 classes were identified; 8 of them were significant, of which only the aforementioned classes contained bacteria with the number in excess of 3.5%. In Group III (double addition of MoO_3NPs), the microbiota of fish intestines contained *Proteobacteria* taxon comprised of two classes—*Alphaproteobacteria* and *Gammaproteobacteria*; however, the number of *Alphaproteobacteria* decreased, whereas the number of *Gammaproteobacteria* is increased by 14.7 ± 0.38% in comparison with Group II. *Firmicutes* taxon included one class: *Bacilli*; its number was increased by 21.68 ± 0.63% in comparison with reference and by 20.26 ± 0.59% in comparison with Group II. In other classes presented by *Actinobacteria* and *Planctomycetia* taxa, the number varied in the range of ± 5% of their number in Group II.

Comparative analysis of microbiota of intestines at the level of families revealed certain differences in reference and experimental groups; thus, at the 7th day of experiment taxonomic analysis revealed 137 and at the 14th day 111 families; however, at the 7th day the numbers of each family prevailed, whereas at the 14th day their diversity increased.

Among the revealed families, *Rhodobacteraceae* family dominated in Group II, whereas the reference group contained only 2.23 ± 0.02%. *Gammaproteobacteria* class was presented by *Xanthomonadaceae* family and *Fusobacteria* class by *Fusobacteriaceae* family. The remaining families, such as *Xanthobacteraceae*, *Moraxellaceae*, *Pseudonocardiaceae*, *Planctomycetaceae*, *Aeromonadaceae* and the most

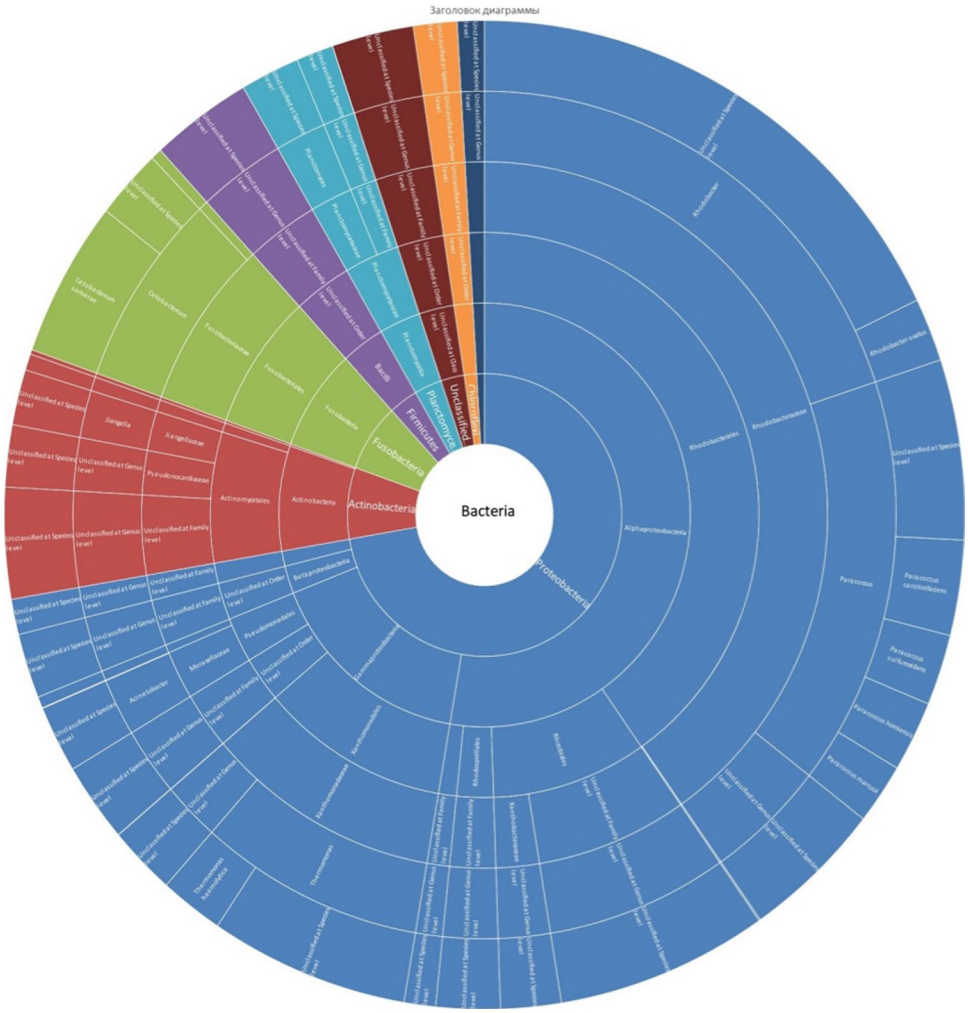

Fig. 3 Taxonomic composition of intestinal microbiota *Danio rerio* of Group II (MoO_3NPs in a dose of 0.2 mg/dm^3, exposure of 7 days)

interesting *Enterobacteriaceae*, dominating in the reference group, were insignificant and amounted to 41.26 ± 1.21% of total value.

In Group III, *Moraxellaceae* family was characterized by the maximum count. The count of *Rhodobacteraceae* family was decreased by 26.77 ± 0.71% in comparison with Group II. Two families were identified in the *Firmicutes* phylum of the *Bacilli* class, which until then had not been identified either in the control or in the II experimental group: *Staphylococcaceae* and *Bacillaceae*.

In total, 273 species were identified in Group II; only four of them were significant: *Rhodobacter*, *Paracoccus*, *Thermomonas* and *Cetobacterium*. Minor taxa (less than 3.5%) were presented by *Acinetobacter* and *Planctomyces*.

Generic composition in Group III slightly varied; *Acinetobacter*, *Staphylococcus*, *Paracoccus*, *Bacillus* and *Rhodobacter* were significant. Other genuses with the count not exceeding 3.5% amounted to 48.55 ± 1.22% of total value.

Species diversity of microbiota of *Danio rerio* intestines at the 7th day of experiment (Group II) was presented by 393 and at the 14th day by 299 morphologically different bacterial species. Herewith, only *Cetobacterium somerae* was significant, non-classified types amounted to more than one-half—55.2 ± 1.23%, and the total amount of non-identified and insignificant species, including less than 3.5%, was 94.91 ± 2.32%. In Group III, *Acinetobacter—A. baumannii*, *A. gerneri*—and *Staphylococcus—S. fleurettii*—were significant.

The study of the microbiocenosis of the gills *Danio rerio*

While studying microbiota of *Danio rerio* gills, it was established that the dominating taxon with occurrence of 98.04 ± 1.61% of total analyzed specimen in reference group I (Fig. 5) was *bacteria*. In this taxon, we identified bacterial species belonging to 19 phyla, the highest count, i.e., above 3.5% of occurrence, was that of *Proteobacteria*, *Firmicutes* and *Actinobacteria*. The remaining phyla, such as *Bacteroidetes*, *Cyanobacteria*, *Chloroflexi* and *Planctomycetes*, were in minority.

Herewith, *Proteobacteria* phylum was presented by three classes, one of which, *Gammaproteobacteria*, occupied

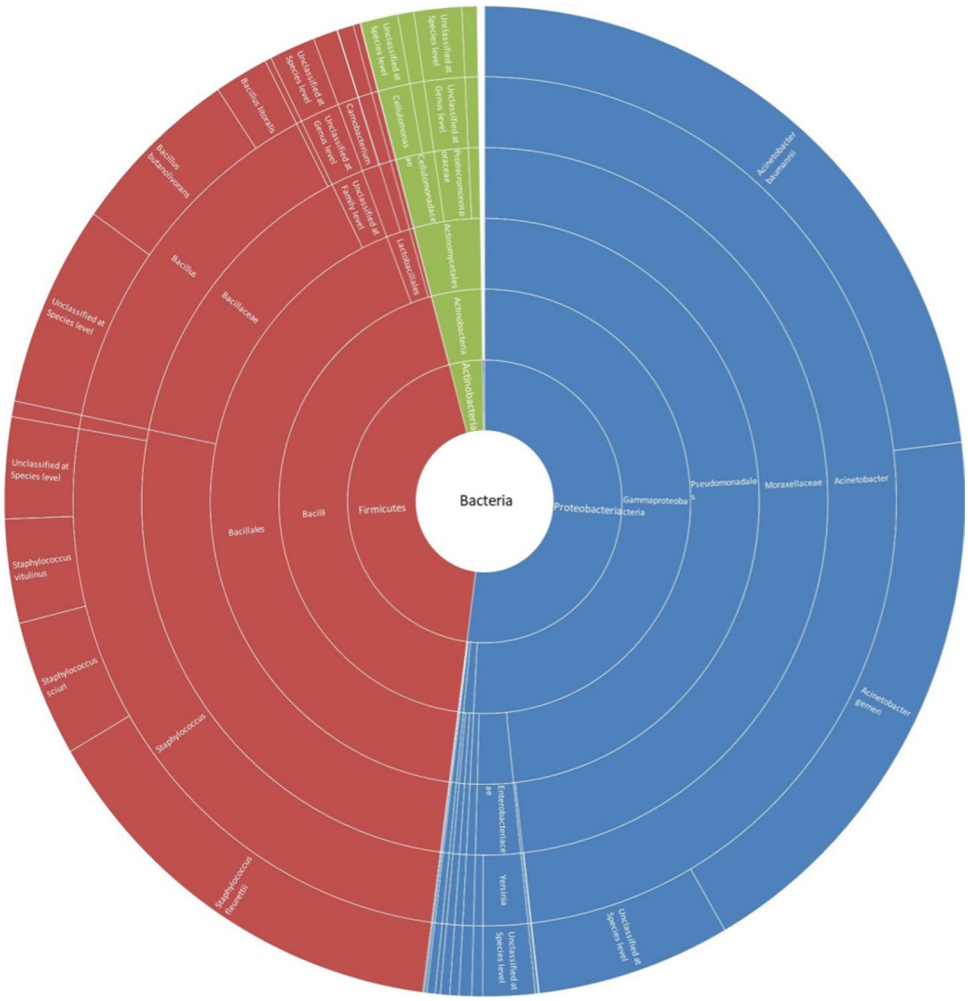

Fig. 4 Taxonomic composition of intestinal microbiota *Danio rerio* of Group III (MoO_3NPs in a dose of 0.4 mg/dm^3, exposure of 14 days)

more than one-half of count of this phylum. Bacterial count of two remaining classes was insignificant, though exceeding 3.5%.

Firmicutes taxon was presented by one class: *Bacilli*, *Actinobacteria* taxon by *Actinobacteria* class.

Upon further analysis of microbiota of *Danio rerio* gills, four families should be mentioned, the count of which exceeded 3.5%. These were *Moraxellaceae*, *Enterobacteriaceae*, *Staphylococcaceae* and *Bacillaceae*. *Moraxellaceae* family was presented by 33.12 ± 0.83%, and together with *Enterobacteriaceae* family (4.68 ± 0.13%), it was referred to *Gammaproteobacteria* class, *Proteobacteria* phylum. Two remaining families, *Staphylococcaceae* and *Bacillaceae*, referring to *Bacilli* class, amounted to 10.70 ± 0.27% and 7.39 ± 0.18%, respectively. Herewith, in *Actinobacteria* class no families were revealed with the count above 3.5%.

Microflora composition of *Danio rerio* gills included 275 genuses and 325 species, 33.10 ± 0.82% were presented by *Acinetobacter*, 10.42 ± 0.28%—by *Staphylococcus*, 7.07 ± 0.18%—by *Bacillus* and 3.79 ± 0.09%—by *Plesiomonas*. Herewith, if *Acinetobacter* genus included two species, *Acinetobacter baumannii* and *Acinetobacter gerneri*, and *Staphylococcus* genus included *Staphylococcus fleurettii* species, then the other genuses were not identified as species.

Group II (Fig. 6) was characterized by the fact that the major portion of revealed bacteria referred to *Actinobacteria*, *Firmicutes* and *Proteobacteria* phyla. Herewith, the addition of MoO_3NPs changed dominating phylum with a certain increase in occurrence of *Actinobacteria* phylum by 44.04 ± 1.09% and decrease in *Proteobacteria* by 35.8 ± 0.83%.

In turn, repeated addition of MoO_3NPs (Group III) (Fig. 7) resulted in reverse structure displacement of gills microbiota with the decrease in the fraction of *Actinobacteria* phylum by 45.27 ± 1.41% in comparison with single addition of MoO_3NPs (Group II) and the increase in the fraction of *Proteobacteria* phylum by 28.27 ± 0.61%. As a consequence, their amount in Group III reached the values of the reference group I. Occurrence of *Firmicutes* phylum tended upward; thus, if in the reference group its content was

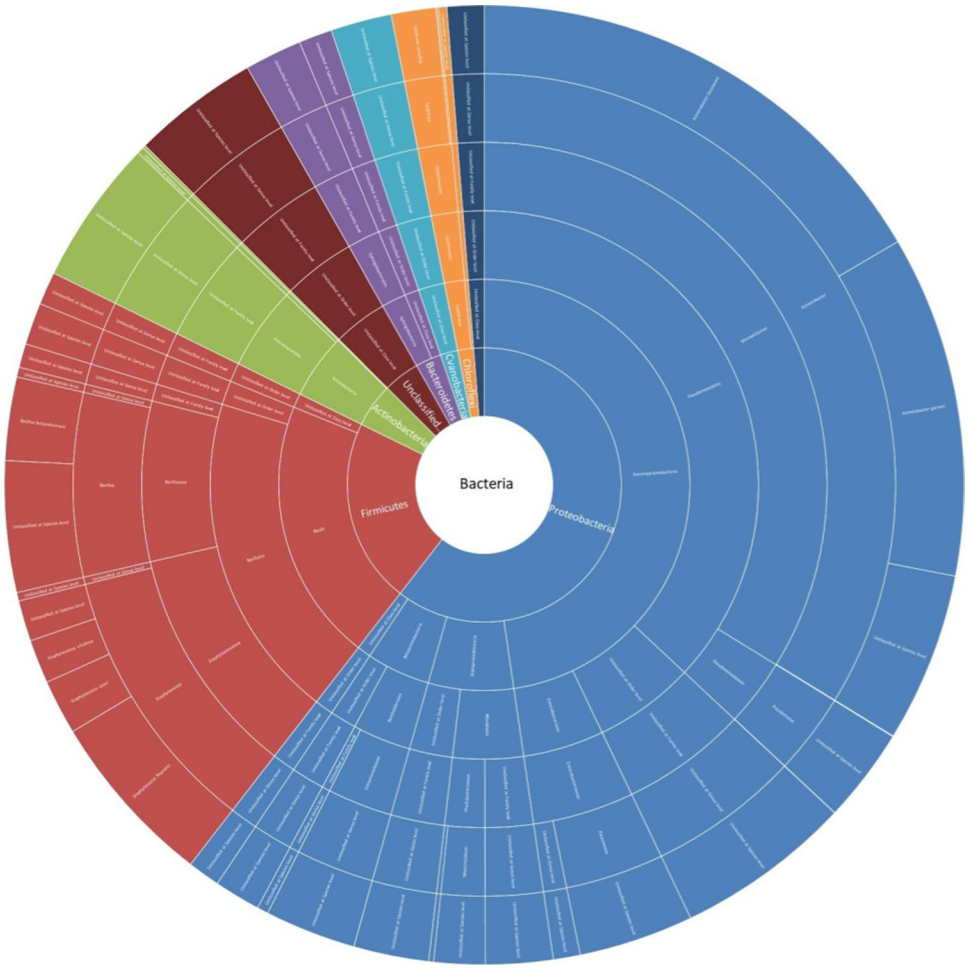

Fig. 5 Taxonomic composition of microbiota of *Danio rerio* gills of the control group I

21.4 ± 0.54%, then in Group II, its content was 25.7 ± 0.65% and in Group III—43.6 ± 1.21%.

And if in Group II *Actinobacteria* phylum was presented by only one class, *Actinobacteria*, *Firmicutes* phylum was also characterized by the existence of *Bacilli* class, and *Proteobacteria* phylum was already presented by two classes: *Gammaproteobacteria* and *Alphaproteobacteria*. Group III was also characterized by the existence of these families; however, *Actinobacteria* amounted to 3.9 ± 0.08%, by 1.13 ± 0.02% lower than in reference group and by 45.27 ± 1.41% lower than in Group II. *Gammaproteobacteria* and *Bacilli* classes in this group dominated, their counts were 50.59 ± 1.28% and 43.54 ± 1.31%, respectively. *Alphaproteobacteria* class became minority, and its count did not exceed 3.5%.

Actinobacteria class identified in Group II was sufficiently homological and presented by two families: *Micrococcaceae* and *Streptomycetaceae*; *Bacilli* class was presented by two classes: *Staphylococcaceae* and *Bacillaceae*, and *Gammaproteobacteria* class—by two families, one of which amounted to 17.09 ± 0.44% (*Moraxellaceae*), and the second one was minority; its count did not exceed 3.5% (*Xanthomonadaceae*). *Alphaproteobacteria* class was sufficiently high, and no families with count in excess of 3.5% were revealed. *Moraxellaceae*, *Staphylococcaceae* and *Bacillaceae* families dominated in microbiota of *Danio rerio* gills. The remaining families were insignificant, and their content varied from 0.83 ± 0.01 to 2.1 ± 0.05%.

Taxonomic diversity at the level of genus in Group II was characterized by 168 genuses; four of them were significant, and their occurrence exceeded 3.5%. They included *Kocuria*, the count of which varied in the range of 41.03–41.87%, *Staphylococcus* and *Acinetobacter* occupied the second position in terms of occurrence, their counts were 18.16 ± 0.46% and 16.95 ± 0.56%, respectively, and *Bacillus* was the most insignificant—5.32 ± 0.14%. Other genuses with the count not higher than 3.5% were presented by *Streptomyces*, *Rhodobacter* and *Thermomonas*. Total content of non-classified genuses was 5.83 ± 0.15%. *Kocuria*, not presented in the reference group in this case, dominated and *Plesiomonas*, presented by 3.79 ± 0.09% in reference group, was not classified in Group II.

In Group III, *Acinetobacter* was present in higher number, and the count of this genus tended upward in comparison

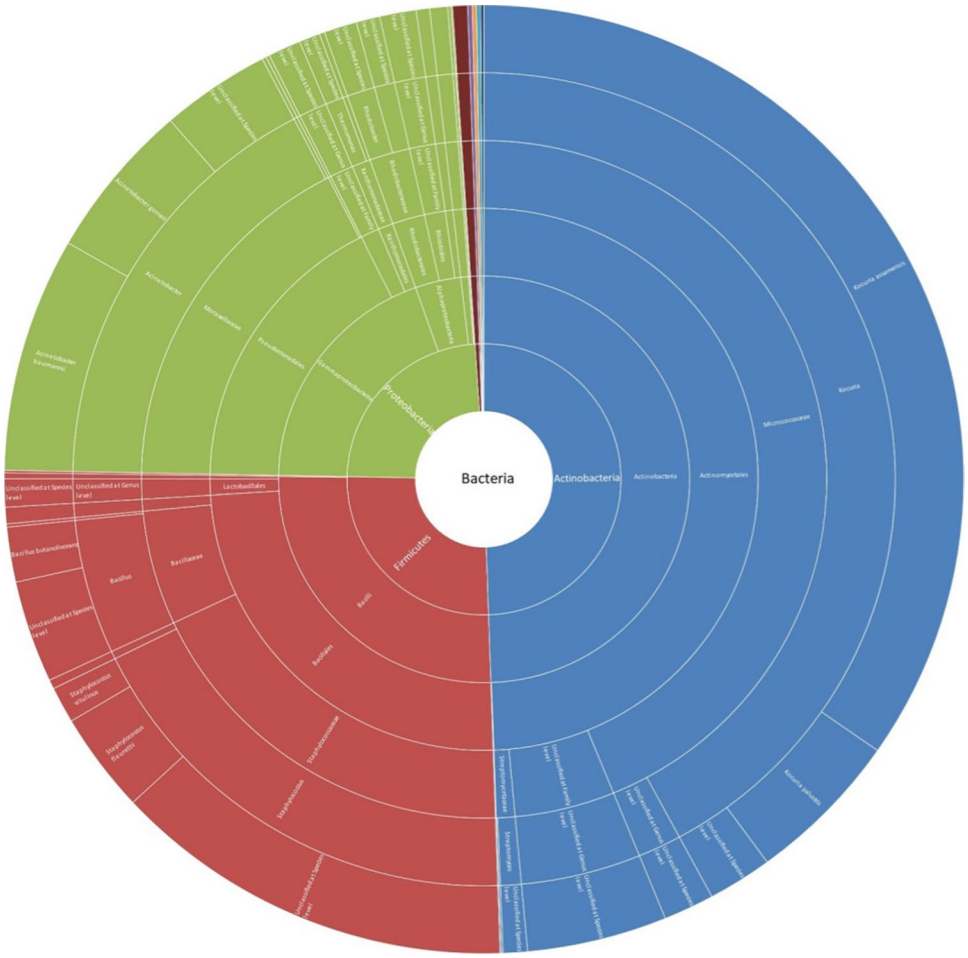

Fig. 6 Taxonomic composition of microbiota of *Danio rerio* gills of Group II (MoO_3NPs in a dose of 0.2 mg/dm^3, exposure of 7 days)

with the reference group. Such situation was characteristic of both *Staphylococcus* and *Bacillus*, and the second and the third identified genuses in terms of count. The remaining identified genuses were minor, and their count did not exceed 3.5%, which evidenced that they were presented in the form of separate isolates.

Diversity of gills microbiota at the 7th day of experiment upon single addition of MoO_3NPs (Group II) included 247 taxonomic units; four species were significant: *Kocuriaassamensis*, *Kocuriapalustris*, *Acinetobacter baumannii* and *Acinetobacter gerneri*. *Staphylococcus fleurettii*, *Staphylococcus vitulinus* and *Bacillus butanolivorans* species were insignificant.

Upon repeated addition of MoO_3NPs at the 14th day of experiment (Group III),142 species were identified, and the counts of each species changed; thus, *Acinetobacter baumannii* became dominant, and *Acinetobacter gerneri* constituted 18.48 ± 0.46%, *Staphylococcus fleurettii*—14.44 ± 0.35%, *Staphylococcus vitulinus*—3.38 ± 0.06% and *Bacillus butanolivorans*—5.74 ± 0.13%. In addition, *Staphylococcus* genus was presented by one more species: *Staphylococcus sciuri* (4.44 ± 0.11%) and *Bacillus* genus—by *Bacillus litoralis* (1.81 ± 0.03%).

Upon generalization of the obtained results, it should be noted that in the reference group 27.49 ± 0.64% were identified by Gram-positive microflora and 11.82 ± 0.28%—by Gram-negative microflora. In the course of experiment, similar ratio of Gram-positive to Gram-negative organisms was retained. Thus, at the 14th day of experiment the fraction of Gram-positive microflora was already 29.81 ± 0.67%.

Discussion

Preparations of molybdenum and its compound classified as nanomaterials are characterized by unique biological properties (Sam et al. 2015; Qureshi et al. 2016) determining a wide range of effects on ecosystems upon their penetration into environment (Kosyan et al. 2015; Rusakova et al. 2015; Lebedev et al. 2016). The obtained results also confirm and agree with the available data (Wang et al. 2012) that the NPs penetrated into organism have influence on established microbial communities continuously by varying both qualitative and quantitative properties of intestines'

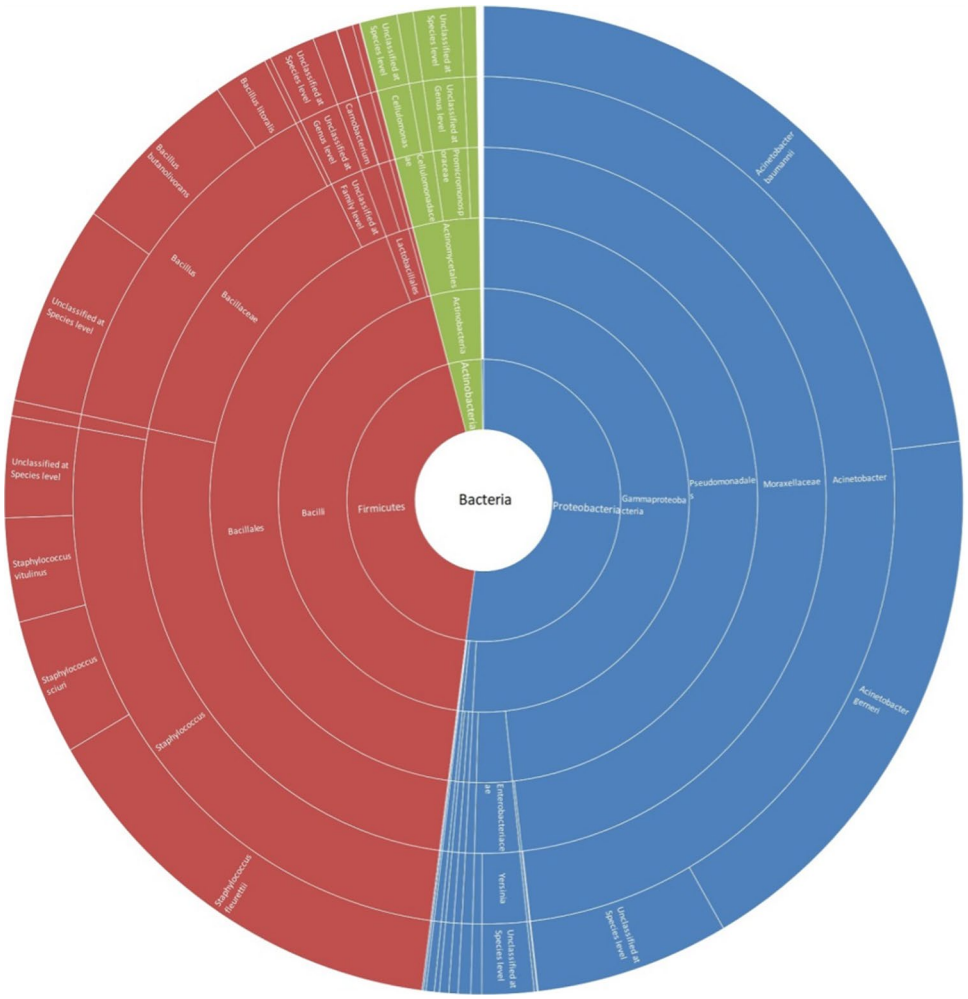

Fig. 7 Taxonomic composition of microbiota of *Danio rerio* gills of Group III (MoO_3NPs in a dose of 0.4 mg/dm^3, exposure of 14 days)

microbiocenosis which in turn influences the overall state of fishes.

Microflora of fish intestines plays an important protecting role in organism (Jankauskiene 2000), and it participates in digestion both in total and in metabolic control (Austin 2002). Qualitative analysis of fish microbiocenosis is very important, since the domination of conventionally pathogenic and pathogenic microflora against deterioration of protecting abilities can result in the initiation of epizooty (Sugita et al. 1992).

According to various estimations, classical approaches are able to cultivate from 3% (Ringo et al. 2001) to 4.8% (Fidopiastis 1996) of total bacterial population from fish intestines, which do not permit to apply conventional methods for sufficient investigation into diversity of existing microflora in intestines (Spanggaard et al. 1993).

The obtained data based on application of 16s rRNA as a marker evidence that the presence of NPs in diets changed the structure of intestinal microbial community. Dominating taxon in microflora of *Danio rerio* intestines was *bacteria*. In the reference group, 18 phyla were classified, in Group II—16 and in Group III—14. With the increase in frequency of the addition of NPs, *Proteobacteria* lose their dominating position in bacterial communities of *Danio rerio* gastrointestinal tract. It should be also mentioned that in our studies *Fusobacteria* were not identified, whereas this phylum occurred sufficiently frequently in intestines of this fish (Roeselers et al. 2011; Lan and Love 2012).

Occurrence of *Staphylococcaceae* in *Danio rerio* gastrointestinal tract was indicated at the decrease in immune forces of organism which often violated digestion and led to pathological processes. In turn, the existence of *Bacillaceae* indicated organism adaptation, modification of microbiocenosis structure (Cantas et al. 2012) related to the addition of MoO_3NPs, attempt to recover normal microflora of intestines and prevention of excessive growth of pathogenic microflora (Kortman et al. 2012).

Conclusion

The obtained results confirm assumption that NPs in digestive tract modify the structure of microbial community (Werner et al. 2011). In particular, some beneficial bacterial strains (e.g., *Cetobacter iumsomerae*) were suppressed to non-detectable levels by NP exposure, involving digestive function and overall health (Merrifield et al. 2013). Close phylogenetic dependence between *Cetobacterium somerae* and *Bacteroides strains*, type A, capable of producing B12 vitamin has been detected (Tsuchiya et al. 2008).

Repeated addition of MoO_3NPs stabilizes microbial community and initiates occurrence of symbionts participating in digestion, including synthesis of biotin (Yossa et al. 2011). However, together with typical interstitial flora of fishes in Group III against double addition of MoO_3NPs occurrence of *Acinetobacter* was identified. Being ubiquists of soil and water media (Chebotar et al. 2014), they initiate infections of gastrointestinal tract and are resistant against antibiotics (Howard et al. 2012).

Changes in phylum ratio, in particular, abundance of *Proteobacteria,* 60.42–93.01%, and relative absence of *Firmicutes* (0–22.63%) can be characterized as exhaustion of anti-inflammatory bacteria leading to decrease in immune response (Natividad et al. 2015). This confirms the necessity to study the contribution by microbial communities into digestion as an indicator of feeding and health of fishes, including popular model organisms (Lammer et al. 2009; Cantas et al. 2012).

The addition of MoO_3NPs transformed microflora, to a greater extent occupying *Danio rerio* gills. Thus, single addition modified dominating phylum with a certain increase in stability of *Actinobacteria* phylum by $44.04 \pm 1.09\%$ and decrease in fraction of *Proteobacteria* by $35.8 \pm 0.83\%$. Such displacement in occurrence of species related to these phyla is attributed to their ecological peculiarities related to the fact that *Actinobacteria* more often dominate at later stages of microbial succession when conditions for the use of difficult-to-access substrates are established or in vitro conditions varying from standard ones (Eduok et al. 2017), which is observed in our case against application of MoO_3NPs. In addition, a peculiar feature of *Actinobacteria*, interacting with eukaryotes, is their ability to synthesis of physiologically active substances of antibiotics assisting the master organism to struggle against unfavorable environmental conditions (Anandan et al. 2016; Mishra et al. 2017).

In the course of studies, $27.49 \pm 0.64\%$ of Gram-positive microflora and $11.82 \pm 0.28\%$ of Gram-negative microflora were identified in the reference group. Similar dependence is observed in the ratio of Gram-positive to Gram-negative microorganisms in experimental groups. Thus, at the 14th day of experiment (Group III) the fraction of Gram-positive microflora was already $29.81 \pm 0.67\%$.

Normal microflora of fish intestines was presented mainly by Gram-negative bacteria, whereas dominance of Gram-positive microflora in Group I could indicate at substitution of autochthonous microflora with allochthonous microflora which was met more frequently in water (Buzoleva et al. 2008). This observation can evidence violation of equilibrium in microbiocenosis of fish intestines and suppression of protecting mechanisms which usually prevent colonization by foreign microflora.

Herewith, it should be mentioned that colonization of gills in this case is related to active ingestion of water, and their bacterial content directly depends on both microbial colonization of water and microorganisms occupying fish intestines. As a consequence, we observe certain homology between identified species in intestines and gills and high coefficients of correlation between occurrences of these species in these two structural areas. To a greater extent, such homology was observed at the 14th day of experiment ($r = 0.939$; $P < 0.005$), when microbial community changed and microorganisms and fish attempted to manage the existence of NPs.

The obtained results demonstrate the influence of MoO_3NPs on changes of biodiversity of *Danio rerio* intestines and gills. NPs exogenically penetrating into organism can destroy the established microbial communities, influence the organism health and suppress protecting mechanisms which usually prevent colonization by foreign microflora.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animals rights All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent Informed consent was obtained from all individual participants included in the study.

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