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Research Article

Bioaccumulation of water-borne silver nanoparticles and silver nitrate in striped catfish, *Pangasianodon hypophthalmus*, fed dietary nucleotides

Behzad POURNORI, Salar DORAFSHAN* & Fatemeh PAYKAN-HEYRATI

Department of Natural Resources, Isfahan University of Technology, Isfahan, 84156-83111, Iran.
**Email:* sdorafshan@cc.iut.ac.ir

Abstract: Recent development in the nanotechnology industry and increase in the number of products manufactured by nanoparticles has inevitably contributed to the discharging of nanomaterials into the aquatic ecosystems. To improve the harmful effects of these toxic materials diverse complements like dietary nucleotides (NT) have been proposed. The effects of dietary NT on silver bioaccumulation in gill and muscle tissues were described after exposure to various concentrations of water-borne silver nanoparticles (AgNPs) and/or silver nitrate ($AgNO_3$) in the present investigation. Specimens of striped catfish, *Pangasianodon hypophthalmus*, were divided into two groups fed with two different diets (control and NTsupplemented 0.75%) over a period of 10 weeks and were subsequently exposed to various concentrations of AgNPs and $AgNO_3$ under static-renewal conditions for 10 days as follow: 1µg/L AgNPs, 1µg/L $AgNO_3$, 20µg/L AgNPs and 20µg/L $AgNO_3$. After the exposure period, samples of gill and muscle tissue were taken to measure silver bioaccumulation via atomic absorption spectrometry. Silver bioaccumulation in gills was about 5-10 times higher than those measured in the muscles. By increasing silver concentrations in the water, its accumulation in the tissues increased significantly. Generally, $AgNO_3$tended to accumulate more than AgNPs in both tissues and all used concentrations. Fish fed on dietary NT showed lower silver accumulation levels in both tissues; although, the lower accumulation was more clear in gills in comparison to the muscles. It could be concluded that adding NT to the diet of striped catfish could significantly boost the fish defense against silver accumulation, but it is recommended to do some detailed studies to find out its mechanism.

Keywords: Atomic absorption, Nanotechnology, Ornamental fish, Toxicity

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Introduction

Nanotechnology is the study and application of material on a nano-scale, and nanoparticles are materials with at least one dimension of 1-100 nanometer. Due to high surface to volume ratio and having 40 to 50 percent of their atoms on the surface, nanoparticles have an unusual potential for reactivity. Therefore, nanoparticles have different physical, chemical and biological properties in comparison to the same materials with larger dimensions (Kreyling et al. 2010). Nano-materials have various industrial applications such as car manufacturing, building materials, electronics, material engineering, food industry, medicine, textile industry, chemistry, cosmetics, household appliances and sporting equipment. Silver nanoparticles, carbon tubes, titanium

oxide, silica, zinc and gold are among the most commonly manufactured nano-materials which have industrial applications (Takenaka et al. 2001). Owing to high toxicity, silver compounds had been widely used as an antibacterial for hygienic applications such as protecting skin injuries from infections (Rai et al. 2009). Although silver nanoparticles have the largest proportion of nano-material production (i.e. roughly 56%) in the world, their adverse effects have been not fully understood (Castellano et al. 2007). Because of the massive production and application of silver nanoparticles and the high likelihood of their release into the aquatic ecosystems, there is a growing concern over the potential risks posed by such materials to living organisms. It has been demonstrated that silver nanoparticles used in textile and plastics account for almost 15% of the overall silver release into the aquatic environments in the European Union (EU) (Blaise et al. 2008). As a result, aquatic nano-ecotoxicology has attracted more attention of researchers as a new field of study. In spite of low silver concentrations in aquatic ecosystems, several studies have pointed out that silver compounds, including silver nanoparticles and silver ions, highly tend to accumulate in the body and cause chronic poisoning in living beings due to their special chemical properties and easy entrance into the body through ion channels and transporters existing in the cell membrane (Benjamin et al. 2011). It is also documented that nano-materials can lead to DNA point mutations and oxidation (Colvin 2003) and hematological and gill histopathological alternation in some fish species (Razmara et al. 2014a,b).

In recent decades, various measures have been taken in aquaculture to reduce the adverse effects resulting from releasing of toxic materials such as silver nanoparticles and silver nitrate into the aquatic environments. Adding anti-stress ingredients such as plant extract or nucleotides (NTs) to the diet of aquatic organisms is an example of such measures (Bahrami Babaheydari et al. 2014; 2015). NTs are cellular components with low molecular weight (on average 325 daltons), are constantly synthesized, decomposed and restored in cells. They have significant effects on growth, cellular metabolism, biochemical and physiological function of cells and almost all cellular reactions; they also play a crucial role in the structural and regulatory functions of the body (Holen & Jonsson 2004; Li & Gatlin III 2006; Yaghobi et al. 2014, 2015a,b). NTs reduce the blood cortisol levels in rainbow trout, *Oncorhynchus mykiss* infected by pancreas necrosis virus (Leonardi et al. 2003) and striped catfish, *Pangasianodon hypophthalmus*, after handling and crowding stress (Yaghobi et al. 2015a) which results from increasing their resistance to disorder factor. The striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) is considered as a species on the verge of extinction in the inland waters of the south-east Asian countries and although it plays a significant role in the aquaculture industry in many countries like Thailand and Vietnam, its wild population is decreasing. Striped catfish has also been introduced to many other countries such as Singapore, USA, the Philippine and Iran as a cultivated food fish or as an ornamental species (Vidthayanon & Hogan 2013).

Silver ions and nanoparticles are categorized as a stress mediated factor. So, it could be assumed that dietary NT can affect silver accumulation in different tissues. Hence, the purpose of this study was to examine the impacts of dietary NT on water-borne silver as nanoparticles or ions on striped catfish, *Pangasianodon hypophthalmus* through assessing the bioaccumulation of silver in the gill and muscle tissues.

Material and Methods

Silver nanoparticles and Silver nitrate. Silver nanoparticles(AgNPs) made by Nanonasb Pars Company under the trade name 'Nanocid' (Tehran, Iran; registration No. 20090013825) with the concentration of4000mg/Lwas used in this study (Table 1, Salari Joo et al. 2013). The

silver nitrate (Merck, Germany) stock solution was prepared using double distilled water with the initial concentration of 100mgL^{-1}. To homogenize AgNPs and silver nitrate solution, sonication was carried out every time just before their application using ultrasonic bath (Micro 10+ sonic, Iran Electronic Industries). Dosage of each tank was adjusted 24 hours before introducing fish to them, to reduce the loss of materials through their sticking to air stones and tank walls (Kalbassi et al. 2012).

Table 1. Some measured specifications of colloidal silver nanoparticles used in this study (Salari Joo et al. 2013). ICP-AES: Inductively coupled plasma-atomic emission spectroscopy; TEM: Transmission electron microscopy.

Parameter	Evaluation method*	Metabolites	Explanations
Concentration (mg/L)	ICP-AES	3980	Concentration with
Shape	TEM	Globular	-------
Particle size	Zetasizer	3.9-163.5 (Av. 83)	54.1% of particles have
The average of the	Zetasizer	54.8	-------
The maximum	TEM	129	65.14% of the particles

Experimental design. Some 80 fish (mean weight and total length 12±0.26g and 10.7±0.46cm) were randomly chosen from each group of fish fed on the control diet and diet containing 7.5g of mixed NT/Kg. The practical diet formulated based on fish meal, soybean oil, soybean meal, corn, corn gluten and fish oil containing 39% crude protein, 14% fat, 21.7% carbohydrate, 3% of fiber and less than 10% moisture (National Research Council (NRC) 1993) was supplemented with the commercial nucleotide mixture "Optimun" (Chemoforma, Augst, Switzerland) to give 0 andOptimun contained inosine monophosphate (IMP), adenosine monophosphate (AMP), guanosine monophosphate (GMP) and ribonucleic acid (RNA). Subsequently, each group was divided into five portions of 16 fish in terms of exposure to silver nanoparticles and ions as follows: control group,1µgL^{-1} silver nitrate ($AgNO_3$), 20µgL^{-1} $AgNO_3$, 1µgL^{-1} AgNPs and 20µgL^{-1} AgNPs. The lower AgNPs concentration was selected to mimic the natural conditions of aquatic environments, to examine their toxic impacts on organisms living in natural habitats (Chae et al. 2009; Scown et al. 2010). To provide equal experimental conditions, both groups of fish were transferred to shared aquariums after marking the caudal fin of those fed with dietary NT. The exposure period lasted for 10 days under the static–renewal conditions during which there was no feeding. Temperature (30±0.2 ºC) was measured via a thermometer; oxygen (6.65±0.55 mgL^{-1}), pH (8.1±0.2) and EC (482.2±10 µs/cm) were measured by DO meter (WTW DO meter, USA), pH meter (Research Jenway 3330) and EC meter (Research Jenway 4310), respectively. Total hardness was measured as 182±14 mg/L $CaCO_3$via titration. The actual concentrations of silver in the aquaria were measured 5 times throughout the exposure period (24h after redoing) by sampling 50ml of the central water column. Atomic absorption spectroscopy (Perkin Elmer Analyst Model A Analyst 700) was used to determine silver concentrations based on the National Institute for Occupational Safety and Health protocol 7300 (NIOSH, 1999) the actual concentrations of silver in 1$AgNO_3$, 20$AgNO_3$, 1AgNPs, and 20AgNPs, were measured to be 1.2±0.02, 19.5±0.05, 1±0.09 and 20.5±0.07µgL^{-1}, respectively. No silver concentration was detected in the control de colorized tap water.

Sampling and accumulation analysis. At the end of exposure (10 days), fish were deeply anesthetized using buffered tricaine methone-sulphonate (MS 222, manufactured by ARGENT Chemical Laboratories, USA) (200 mgL^{-1}). The gill and muscle tissues of at least 8

individual fish were collected from each treatment, were weighed and placed at 60 º C for 48h.Tissue digestion was done at 125º C for 24 h using 4mL nitric acid and 1mL hydrogen peroxide (Sigma-Aldrich, France). Temperature was then increased to 190 ºC and samples were subsequently dissolved in 10mL nitric acid (10%) and 200µL triton X100 (10%). Samples were analyzed using the atomic absorption spectrometer (Perkin Elmer Analyst Model A Analyst 700) on a wavelength of 328.1 nanometer.

Statistical analysis. Data are expressed as an average ± standard error of mean (S.E.M). Statistical analysis was performed through SPSS 19 software at the significance level of 0.05. The data normality was examined by means of Kalmogorv-Smiranov Test. After confirming their normality, data were analyzed using One-way ANOVA test. The independent *t*-test was used to determine whether there is a statistically significant difference between the means in two groups of fish fed with and without NT in the same concentration of individual chemical, e.g. 20µgL^{-1} AgNPs. Moreover, comparisons were made between identical tissues from different treatments using Duncan multiple range test (DMRT) at a significant level of 0.05.

Results

In general, silver concentrations in the tissues (gill and liver) of the fish after exposing to the decolorized tap water was too low (Figs. 1,2). It was also indicated that silver accumulation was much higher (about 10 folds) in the gill compared to muscle tissues, regardless of chemicals kind and/or their concentrations (Figs. 1,2), showing that the gills were the major border against water-borne silver. It was also clearly demonstrated that the higher doses of water-borne AgNPs or $AgNO_3$, 20µgL^{-1}, caused higher accumulation in the tissues in comparison to lower doses of chemicals e.g., 1µgL^{-1} (Figs. 1,2). In both tissues, the highest accumulation was detected when the fish were exposed to the highest level of $AgNO_3$ (20µgL^{-1}), followed by highest dose (20µgL^{-1}) of AgNPs (Figs. 1, 2; $P < 0.05$).

Silver bioaccumulation in the gill tissue showed that dietary nucleotide could lead to lower accumulation when fish exposed to 20$AgNO_3$, 1AgNPs or 20AgNPs in comparison to those fish fed on the control diet (Fig. 1; $P<0.05$). No significant differences were observed between groups of fish fed on control or nucleotide added diet when the fish exposed to the lowest dose of $AgNO_3$ (1µgL^{-1}) or decolorized tap water (Fig. 1; $P>0.05$). Fish fed on the control diet showed the highest silver accumulation in the gill tissue (4.9±0.13 µg g^{-1}) when they exposed to 20$AgNO_3$ followed by 20AgNPs (2.47±0.1 µg g^{-1}) (Fig. 1; $P<0.05$). In this group of fish, exposure to 1AgNPs or 1$AgNO_3$ did not cause any significant changes in the silver accumulation in the gills (1.24 vs 1.47µg g^{-1}; Fig. 1; $P>0.05$).

Similar patterns were observed in the gill of fish fed on the dietary nucleotide (Fig. 1); where the highest silver concentration were detected in the fish faced to the 20$AgNO_3$, then 20AgNPs (3.34±0.82 and 1.94±0.98µg g^{-1} respectively Fig. 1). The fish which has been subjected to 1$AgNO_3$ or 1AgNPs showed significantly lower levels of silver concentration (0.93±0.17 or 0.85±0.70 µg g^{-1} respectively; Fig. 1; $P<0.05$). As indicated before, silver concentration in the muscle tissues was too low ranged 0.28-0.72 µg g^{-1} (Fig. 2). In comparison between two groups of fish fed on the dietary nucleotide or control diets, the only significant difference in the silver concentration was detected when the fish were exposed to the highest dose of $AgNO_3$, 20 µgL^{-1} (Fig. 2; $P<0.05$). The fish fed on the control diet tended to accumulate up to about 2 folds higher silver (0.72 vs 0.46µg g^{-1}) in comparison to those of fish fed on the dietary supplemented diet (Fig. 2; $P<0.05$). No significant differences were detected in silver accumulation in the fish exposed to other levels of AgNPs or $AgNO_3$ (Fig 2; $P>0.05$).

For the group of fish fed on the control diet, the highest levels of silver concentration were

observed in the fish faced to $20AgNO_3$ followed by 20AgNPs (Fig. 2; $P<0.05$). No differences were detected when the fish exposed to $1AgNO_3$ or 1AgNPs and the silver concentration in the muscle tissues in these groups were ranged 0.30-0.32 µg g^{-1} (Fig. 2; $P>0.05$).

For the group of fish fed on the dietary nucleotide, the results were as follow: the highest level of silver in the muscle was detected when fish exposed to $20AgNO_3$ or AgNPs (0.46±0.029 or 0.39±0.04 µg g^{-1}) which were significantly higher than the fish exposed to $1AgNO_3$ or control tap water (Fig. 2; $P<0.05$). Interestingly, exposing fish to 1 or 20 AgNPs did not affect silver bioaccumulation in the muscle tissue this group of fish (Fig. 2; $P>0.05$).

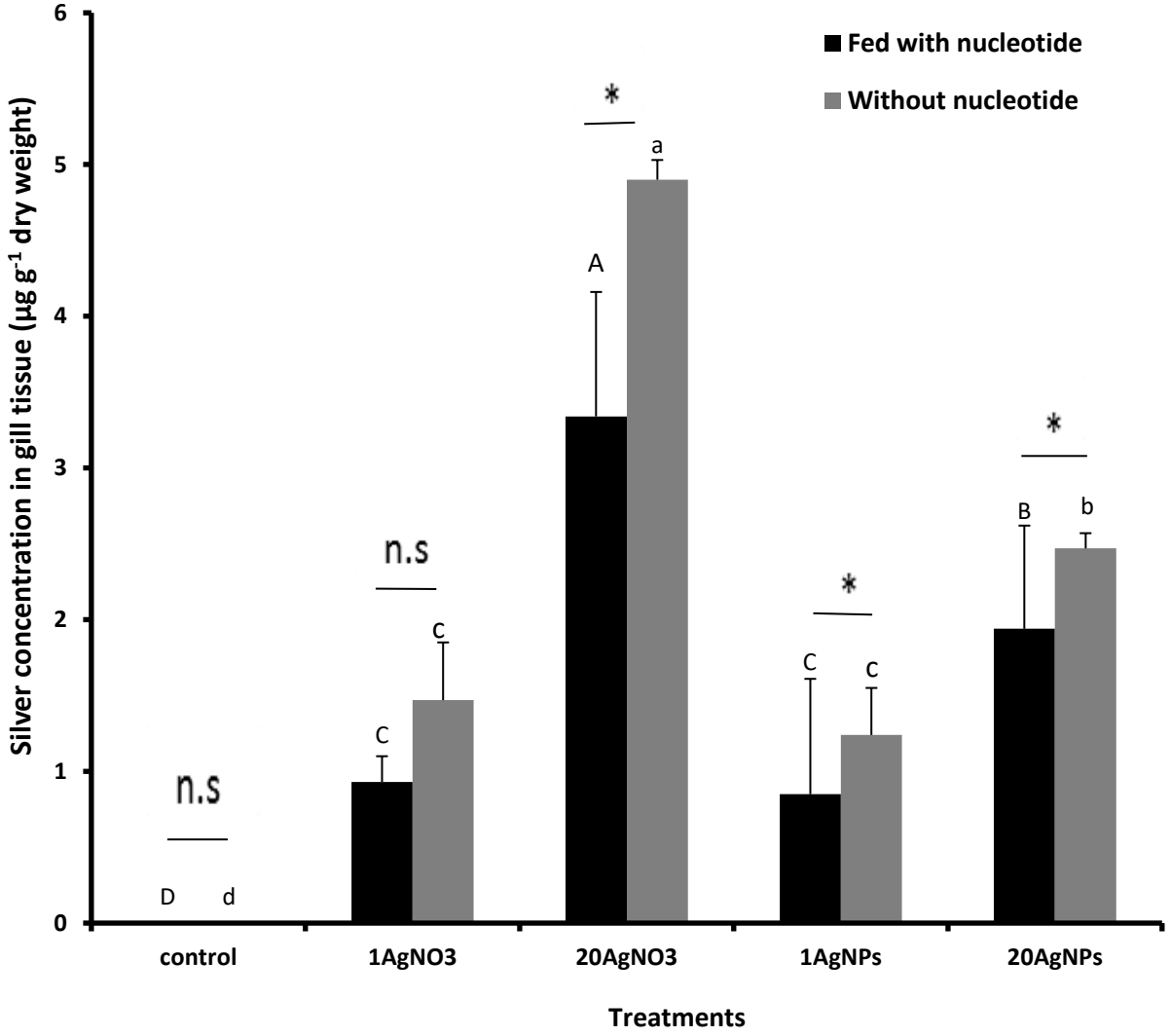

Fig. 1. Silver concentration of gill tissues in the fish fed on dietary nucleotide or control diet exposed to different doses (1 or 20µg/L) of silver nitrate ($AgNO_3$) or silver nanoparticle (AgNPs) or decolorized tap water (control). The lower and upper case letters have been used to represent the significant difference within group of fish fed on control or dietary enriched nucleotide respectively. The existence of at least one similar letter shows a lack of significant difference ($P> 0.05$). *and n.s are used to show significant and non-significant differences between two groups of fish in a same treatment, e.g. $20AgNO_3$. Data is shown as mean±SE.

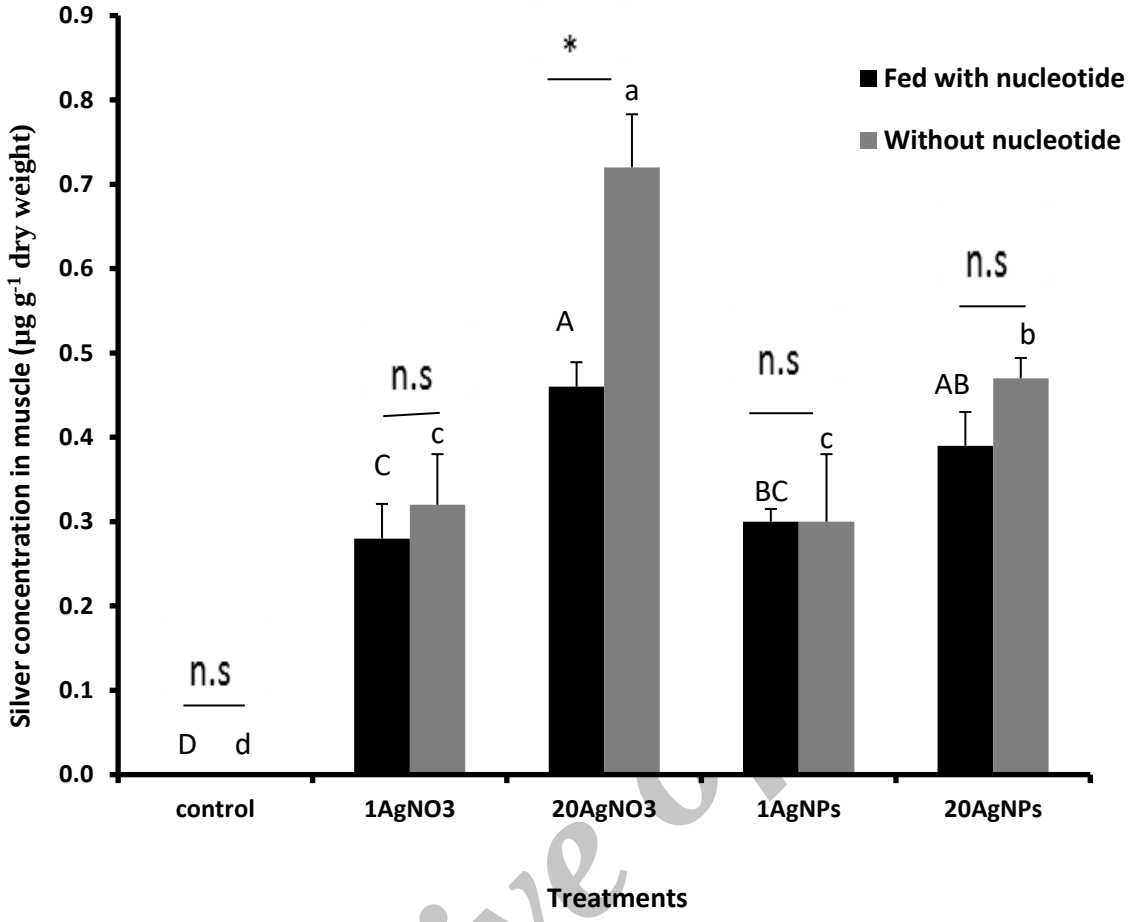

Fig. 2. Silver concentration of in the muscle tissues in the fish fed on dietary nucleotide or control diet exposed to different doses (1 or 20 μgL^{-1}) of silver nitrate ($AgNO_3$) or silver nanoparticle (AgNPs) or decolorized tap water (control). The lower and upper case letters have been used to represent the significant difference within group of fish fed on control or dietary enriched nucleotide respectively. The existence of at least one similar letter shows a lack of significant difference (P> 0.05). *and n.s are used to show significant and non-significant difference between two groups of fish in a same treatment, e.g. $20AgNO_3$. Data is shown as mean±SE.

Discussion and Conclusions

The bioaccumulation of different chemicals including heavy metals and nanoparticles are affected by several factors including exposure time (duration), chemical type, concentration, exposure rut or method of administration (bath or feeding). In this study, gill tissues tended to accumulate more silver in comparison with the muscle tissue regardless of kind of chemicals and their concentration. There are diverse reasons for this issue and one of the most significant ones is that gills are regarded as the first barrier against many different components and contamination in the water and accumulate them very actively to reduce the danger of their transportation to other sensitive organs such as brain, bone and liver (Shaw et al. 2012). Besides, it is expressed that in the freshwater living things since gills are considered as the main water entrance rout, therefore, it can be expected that accumulation in gills become much higher than the other organs (Orojali et al. 2014). In contrast to the freshwater fish, fish living in the saline or marine waters drink water very actively and the higher concentration of such a heavy metals are measurable in the intestine and kidney instead of gills (Kim et al. 2004). Furthermore, it is stated that in teleost

fish metals generally accumulate in the gills as the central internal compartment (Shaw et al. 2012). Another reason could be the lack of metal-binding proteins in other tissues such as muscles (Wang et al. 2014). It is also clearly demonstrated that at the end of exposure period, silver bioaccumulation in fish exposed to $AgNO_3$ was higher than those exposed to silver nanoparticles. The low bioavailability of metals in the nanoparticle forms in gills could be attributed to nanoparticles refinement by the gill mucous caused scouring and absorbing them before getting to the muscles. However, to cope with ions like Ag^{2+} derived from $AgNO_3$, which could enter very actively via gills into the ion regulation processes; this mechanism is more complicated and rarelyhappens. Similar reports are also based on ion accumulation in comparison to nanoparticles not only about silver but also regarding other metals such as copper in different fish species including rainbow trout and orange-spotted grouper, *Epinephelus coicoides* (Shaw et al. 2012; Sovova et al. 2014; Wang et al. 2014).

Also, in this investigation on the highest silver concentration was observed in $20AgNO_3$treatment in fish fed on control diet (non-enriched with NT).It seems that dietary NT can prevent silver bioaccumulation in striped catfish tissues. It could be assumed that dietary NT could affect mucus secretion by gill epithelial layer which could reduce toxin (for example metals) absorption and accumulation by fish. Yaghobi et al. (2015b) opined that dietary NT could increase some intestine properties such as fold and entrocyte heights in the catfish which attributed to the increasing number of intestine cell wall including goblet cells which are agents for mucus secretion. It is also documented that dietary NT could improve intestinal cell growth which probably indicate the role of these compounds in increasing epithelial layer growth in the gill (Holen & Jonsson 2004). Another possible explanation would be higher capacity of gill cells to expulse silver in fish fed on dietary NT in comparison to those fed on the control diet. After all, the matter remains far from clear before adequate study is carried out to shed more light on the specific effects of NT on metal accumulation. There has not been yet a precise explanation regarding the effects of dietary NT on heavy metals bioaccumulation in fish, it has been stated that diets containing NT could improve growth performance, osmoregulation capacity and stress resistance in different fish species such as Atlantic salmon, *Salmo salar*, rainbow trout, channel catfish, *Ictalurus punctatus* and striped catfish (Burrells et al. 2001; Welker et al. 2011; Palermo et al. 2013; Yaghobi et al. 2015a,b).It could be concluded that although exposing striped catfish to both silver nanoparticles and silver nitrate (in 1 and 20µg/L concentrations) led to silver bioaccumulation in gill and muscle tissues, the bioaccumulation of silver in gill tissue was by far higher, mainly in silver nitrate treatment. Additionally, dietary NT could at least to some extend prevent silver bioaccumulation in striped catfish tissues which may indicate the appropriateness of the use of NT in some aquatics.

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مقاله پژوهشی

تجمع زیستی نانوذرات نقره و نیترات نقره محلول در آب در گربه‌ماهی نواری، *Pangasianodon hypophthalmus*، تغذیه شده با نوکلئوتید

بهزاد پورنوری، سالار درافشان* و فاطمه پیکان حیرتی

گروه شیلات، دانشکده منابع طبیعی، دانشگاه صنعتی اصفهان، ۸۳۱۱۱-۸۴۱۵۶، اصفهان، ایران.
*Email: sdorafshan@cc.iut.ac.ir

چکیده: توسعه اخیر در صنعت نانوفناوری به همراه افزایش تعداد محصولات تولید شده با نانوذرات، احتمال رهایش آنها را در اکوسیستم‌های آبی افزایش می‌دهد. به منظور بهبود اثرات مضر این مواد سمی، استفاده از ترکیبات متنوعی نظیر نوکلئوتیدها پیشنهاد می‌شود. در این مطالعه، اثر جیره حاوی نوکلئوتید بر تجمع نقره در بافت آبشش و کبد پس از ده روز مواجهه با غلظت‌های مختلف نانوذرات نقره (AgNPs) یا نیترات نقره ($AgNO_3$) مورد ارزیابی قرار گرفت. گربه‌ماهیان نواری *Pangasianodon hypophthalmus* در دو گروه با دو جیره مختلف شاهد و حاوی ۰/۷۵٪ نوکلئوتید به مدت ۱۰ هفته تغذیه شدند و پس از آن با غلظت‌های مختلف نانوذرات یا نیترات نقره محلول در آب تحت شرایط ساکن-تجدید به مدت ده روز مواجه شدند: شاهد، غلظت ۱ میکروگرم در لیتر از نانوذرات یا نیترات نقره و غلظت ۲۰ میکروگرم در لیتر از نانوذرات یا نیترات نقره. پس از دوره مواجه‌سازی، نمونه‌هایی از بافت آبشش و عضله ماهیان جمع‌آوری و غلظت نقره در آن با استفاده از روش اسپکتوفتومتری جذب اتمی سنجش شد. تجمع نقره در بافت آبشش حدود ۵ تا ۱۰ برابر بیشتر از بافت عضله بود. با افزایش غلظت ترکیبات نقره در آب، میزان تجمع آن در بافت‌ها به طرز محسوسی افزایش یافت. به طور عمومی، نیترات نقره تمایل بیشتری به تجمع در بافت‌ها در مقایسه با نانوذرات نقره در تمامی غلظت‌های مورد مطالعه نشان داد. تغذیه ماهیان با جیره حاوی نوکلئوتید منجر به کاهش تجمع نقره در هر دو بافت مورد بررسی شد، اگرچه تجمع کمتر در بافت آبشش در مقایسه با عضله مشهودتر بود. بر اساس نتایج می‌توان بیان داشت که افزودن نوکلئوتید به جیره گربه‌ماهی نواری می‌تواند منجر به افزایش دفاع ماهی علیه تجمع نقره شود، با این وجود برخی مطالعات تکمیلی در خصوص درک چگونگی این فرایند پیشنهاد می‌شود.

کلیدواژه‌ها: جذب اتمی، سمیت، ماهی زینتی، نانوفناوری