Barbel (Barbus barbus callensis) sperm parameters and oxidative stress status as bioindicators of freshwater pollution

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Abstract

The purpose of this study was to investigate the effects of polluted Soummam River and unpolluted Agrioun River on sperm parameters and oxidative stress status of *Barbus callensis* spermatozoa during the spawning season in natural condition. The experimental design consisted to activate alternatively fish sperm of the two sites with the polluted (Soummam River, S) and unpolluted water (Agrioun River, A). Sperm motility duration (SMD) was measured using a stopwatch. Gametes straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), spermatozoa concentration (SC), straightness (STR) and linearity (LIN) were measured by a CASA. Oxidative stress biomarkers were evaluated by measuring total antioxidant status (TAS) and catalase (CAT) activity. The results showed that the SMD and spermatozoa velocity were significantly higher in (Sm, S) than in (Ag, A) with SMD = 52 versus 42s, VSL = 23 versus 16 µm/s, VAP = 35 versus 25 µm/s, and VCL = 52 versus 35 µm/s, respectively. However, SC, STR and LIN were significantly higher in (Ag, A) than in (Sm, S) with SC = $37.5x10^9$ versus $27x10^9$ spz/ml, STR = 52 versus 40% and LIN = 35 versus 26%. Likewise, the oxidative status of fish spermatozoa was significantly affected by the quality activating water; TAS and CAT were significantly higher in (Ag, A) than in (Sm, S); 7.5 to 0.5 and 120 to 28 µmol/min/ml, respectively. The current investigation showed that *Barbus callensis* sperm motility parameters, particularly spermatozoa concentration, straightness and linearity are good bioindicators of water pollution.

Introduction

Over recent decades, aquatic environments are continuously being contaminated with hazardous pollutants originating from domestic sewage, wastewater treatment plant effluent, and industrial and agricultural activities (Bernhardt et al. 2017; Peng et al. 2018; Chen et al. 2019; Moore and Bringolf 2020). In naturel conditions, aquatic organisms, including fish, absorb the pollutants from water and from food chains (Guzzetti et al. 2018; Luczynska et al. 2018) which can affect male reproductive system (Ul Islam et al. 2017), endocrine system (Cao et al. 2019) growth and metabolism (Zebral et al. 2018), antioxidant system and genetic parameters (Jiang et al. 2015), behavior (Kim et al. 2014), plasma membrane (Dasmahapatra et al. 2019) and semen parameters (Kollar et al. 2018).

Recently, there are many publications in which fish was used as a bioindicator of environmental pollution (Luczynska et al. 2018; Santana et al. 2018; Cerveny et al. 2016; Hussain et al. 2018; Calado et al. 2020; Bernal-Rey et al. 2020). In addition, there are several reasons for the use of fish for evaluating of environmental contamination as they are very sensitive biomarkers to pollutants-induced damage (Bernal-Rey et al. 2020) and a good biological membrane model to analyze oxidative stress (Kollar et al. 2018). Particularly, fishes are indicated animal models for genotoxicological studies, since they have been used over the years due to their fast reproductive cycle (Dasmahapatra et al. 2019).

Several studies have shown that sperm parameters, especially motility duration, gametes concentration and velocity vary significantly through the spawning season in freshwater fish (Aberkane et al. 2018). Similarly, it is shown that sperm quality is significantly related to motility quality (Kime et al. 2001; Au et

al. 2002; Rurangwa et al. 2004) and significantly affected by pollutants (Goncalves et al. 2018; Xiang et al. 2019; Jenkins et al. 2018). Principally, these pollutants affect the oxidative balance which could directly disrupters the reproductive success of fish (Paravani et al. 2019; Persch et al. 2018; Lin et al. 2018) by generating reactive oxygen species (ROS) in sperm cells. This causes loss of viable spermatozoa and motility, lipid membranes peroxidation and infertility (Billard et al. 1995; Ulloa-Rodriguez et al. 2017; Sadeghi et al. 2018).

According to recent data, the Soummam River is an extreme polluted urban River in Bejaia (Algeria) due to increased urbanization process, and industrial and agricultural activities (Maane-Messai et al. 2010; Djoudad-Kadji et al. 2012; Aberkane 2016; Khebbache et al. 2017). In contrast, Agrioun River is an unpolluted aquatic ecosystem (Aberkane and Iguer-ouada 2011).

Barbus callensis is an endemic freshwater species abundantly distributed throughout the Northern African region (Kara 2012) and at today, little is known about the effect of pollution on sperm quality during the spawning season in natural condition. This study aimed at analyzing the impact of water origin from polluted/unpolluted River on Barbel sperm with a special focus on the effect on sperm parameters (SMD, sperm velocity, SC, STR, LIN), including oxidative stress status, considered as a good bioindicator of early warning to reproductive disorders in fish.

Material And Methods

Fish handling and gamete collection

The barbel (*Barbus barbus callensis,* Cyprinidae family) was collected from two aquatic ecosystems, Soummam River (S1: 36° 34’ 42.5” N/5° 4’ 37.3” E) and Agrioun River (S2: 36° 38’ 31.3” N, 5° 20’ 21.2” E) during the spawning season between March and August 2016. The fish were captured using fishing rod connected to a lift net, 2 cm mesh size. Altogether, 171 specimens of male *Barbus callensis* were captured. The fish were brought to the laboratory alive, after drying and cleaning the genital papilla with a paper towel to prevent water contamination and initiation of sperm motility, a gentle abdomen pressure was applied to collect semen. The samples contaminated with the faeces and urine were discarded (Gallego et al. 2013).

Sperm analysis

Sperm samples Ag (Agrioun) and Sm (Soummam) were diluted 1/1000 (activated) alternatively with the water from the both origin: Agrioun water (A) and Soummam water (S). Sperm motility duration (SMD) was assessed using a stopwatch; sperm was considered as immotile when less than 5% of spermatozoa remained motile (Tuset et al. 2008). The parameters obtained by the computer-assisted sperm analysis (CASA) (SCA, 4.0, 2014) software (Microptic S.L.; Barcelona, Spain) were as follows: spermatozoa concentration (SC), velocity straight line (VSL), velocity average path (VAP), velocity curvilinear (VCL), straightness (STR, %), defined as the ratio VSL/VAP and linearity (LIN, %), defined as the ratio VSL/VCL.

Biochemical analysis

Total antioxidant status (TAS)

The total antioxidant status (TAS) was determined using the method of Re et al. (1999). This method involved a direct production of the blue/green ABTS+ [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] radical chromophore through the reaction between ABTS and potassium persulphate. Briefly, after overnight incubation, the colored solution was diluted with phosphate-buffered saline (PBS) (pH 7.4) until the absorbance of 0.7 (± 0.02) was observed at 734 nm using a spectrophotometer. Finally, 2 mL of diluted ABTS°+ was added to 20 µL of each sample in PBS and the absorbance was noted exactly 6 min after initial mixing.

Catalase activity (CAT)

Catalase activity was measured by adding hydrogen peroxide ($H_2O_{2)}$ to the samples and following its decomposition over time by 240 nm absorbance (Aebi 1984). 20 µL of the supernatant was mixed with 1255 µL of phosphate buffer (50 mM, pH 7.0) and the reaction was started by adding 725 µL of H_2O_2 (54 mM) at 25°C for 1 min. The blanks contained 20 µL of supernatant and 1980 µL of phosphate buffer (50 mM, pH 7.0). CAT activity per min was calculated using the molar extinction coefficient (=43.6 l/mol cm) and the results are presented in µmol/min/mL.

Statistical analysis

Statistical analysis was performed using Stat view 5.0 software (Abaccus). All experiments were repeated at least three times. All variables were expressed as the mean ± standard error. To evaluate the differences between semen parameters and oxidative status of *B. callensis* spermatozoa from an Agrioun unpolluted River (Ag) and Soummam polluted River (Sm) activated alternatively with Agrioun water (A) and Soummam water (S), in all the analysis an ANOVA (One-way Analysis of Variance) was performed for normally distributed variables, followed by post hoc Tukey's HSD test ($P < 0.05$).

Results

The relationship between sperm motility duration (SMD) and fresh water quality are presented in Fig. 1. The SMD was significantly higher in sperm from Soummam polluted River activated with the water of the same origin (Sm, S) compared to sperm from unpolluted Agrioun River activated with Agrioun water (Ag, A) with 52 s and 42 s, respectively.

Interestingly, SMD decreased when Ag sperm was activated with Soummam polluted water (Ag, S; from 42 to 38s), and similarly SMD decreased when Soummam sperm was activated with Agrioun unpolluted water (Sm, A; from 52 to 45s). All gametes velocities showed lower velocities when sperm from Agrioun River is activated with the water of the same origin (Ag, A) (VSL = 16µm/s, VAP = 25µm/s and VCL = 35µm/s, Fig. 2). Sperm from Soummam River activated with Soummam water (Sm, S) presented the

highest values with: VSL = 23µm/s, VAP = 35µm/s and VCL = 52µm/s. Also, the velocities of Ag sperm increased significantly when activated with Soummam polluted water (Ag, S) with: VSL = 20µm/s, VAP = 30 µm/s and VCL = 45 µm/s. Likewise, velocity of Soummam sperm decreased significantly when activated with unpolluted Agrioun water (Sm, A) with: VSL = 17µm/s, VAP = 25 µm/s and VCL = 35 µm/s. In contrast to SMD and gametes velocities, spermatozoa concentration was higher in Agrioun unpolluted River (Ag, A; $37.5x10^9$ spz/ml) compared to Soummam River (Sm, S; $27x10^9$ spz/ml) (Fig. 3). STR and LIN (Figs. 4 and 5) showed highest values in Agrioun sperm activated with Agrioun water (Ag, A) compared to Soummam sperm activated with Soummam water (Sm, S) with: STR = 52 versus 40%, LIN = 35 versus 26%. Systematically, STR and LIN decreased when Agrioun sperm is activated with Soummam River (Ag, S): STR from 52 to 39%, LIN from 35 to 31.5% and increase when Soummam sperm is activated with Agrioun River water (Sm, A): STR from 40 to 53% and LIN from 26 to 37%.

Total sperm antioxidant status (ABTS°+ scavenging activity) and catalase activity are presented in Figs. 6 and 7, respectively. Both ABTS°+ scavenging activity and catalase activity were significantly higher in (Ag, A) than (Sm, S) (p < 0.0001). The antioxidant activity of fish spermatozoa collected from the Agrioun River and activated with unpolluted Agrioun fresh water (Ag, A) was 7.5, presented important antioxidant activity when compared to the spermatozoa collected from the Soummam River and activated with polluted Soummam fresh water (Sm, S) was 0.5 (p < 0.0001). Also, the antioxidant activity of fish spermatozoa collected from the Agrioun River and activated with polluted Soummam fresh water (Ag, S) decrease to 1.8 and the antioxidant activity of fish spermatozoa collected from the Soummam River and activated with unpolluted Agrioun fresh water (Sm, A) increase to 2. Similarly, catalase activity in (Ag, A) and (Sm, S) was 120 and 28 µmol/min/ml, and decrease to 20 in (Ag, S) and increase to 50 µmol/min/ml in (Sm, A) (p < 0.0001), respectively.

Discussion

The aquatic ecosystems is the most endangered portion of the Earth's biosphere as it is the final destination of most of the pollution produced by humans (Servili et al. 2020). In Algeria, aquatic ecosystems contaminants have been identified since the mid-1990's, which likely contributed to decrease the freshwater fish population (Djoudad-Kadji et al. 2012; Aberkane et al. 2018; Khebbache et al. 2017). Previous studies reported that sperm quality usually refers to the motility, especially, sperm motility duration and gametes velocity (Billard et al. 1995; Linhart et al. 2000; Alavi and Cosson 2006) is a prerequisite factor determining fertilizing ability (Billard 1978; Cosson et al. 1991; Gallego and Asturiano 2019). Similarly, it is well known that sperm motility in fish is dramatically affected by pollution (Kovacik et al. 2018; Hayati et al. 2019; Kowalska-Góralska et al. 2019). The current results showed that the highest SMD and sperm velocity (VSL, VAP and VCL) were observed in Soummam polluted River (Sm, S). This is similarly reported in Sea trout (*Salmo trutta m. trutta* L.), where the duration of motility increased under copper pollution (Kowalska-Góralska et al. 2019). Also, increased sperm velocity is observed in rats exposed to endocrine disruptors (nonylphenol and atrazine) (Duan et al. 2016; Chen et al. 2019). However,

several studies showed that the pollution decreases motility parameters including SMD and gametes velocity (Shaliutina et al. 2017; Silva Pinheiro et al. 2020).

Under naturel condition, fish semen has molecular inhibitors whose roles include regulation of spermatogenesis, stimulation of sperm velocity and removal of damaged and immature sperm (Alavi and Cosson 2006). Results of increasing SMD and sperm velocity in fish captured from polluted water (Sm, S) may be due to the deregulation of such underlying mechanisms. It is reported that high temperature and pH increase sperm motility in mosquitofish males (*Gambusia holbrooki*) (Adriaenssens et al. 2012). In our study area, the temperature and pH (28°C, 8.22) in Soummam River is higher than in Agrioun River, (20°C, 7.5), factors that could be involved in enhancing gametes motility parameters (Aberkane 2016). Sperm quality could be defined as the ability of the spermatozoa to exploit their swimming ability to reach and fertilize the oocyte (Fauvel et al. 2010). Under environmental pollution, the trajectory of spermatozoa can become increasingly curved and eventually become tight concentric circles (Rurangwa et al. 2004). This is observed in the current study with the lowest values for straightness (STR) and linearity (LIN) when gametes from Agrioun and Soummam River are activated with Soummam polluted water. Spermatozoa concentration (SC) is a useful biomarker to measure sperm quality in fish (Fauvel et al. 2010; Rurangwa et al. 2004). In the current study sperm from Soummam polluted River showed 10^9 spz/ml lower than sperm from Agrioun River. Recently, such spermatozoa concentration declining is reported under water pollution (Silva Pinheiro et al. 2020). The current findings are also in agreement with studies on common carp (*Cyprinus carpio*) (Lugowska 2018) and Zebrafish (*Danio rerio*) reporting lower spermatozoa concentration under exposure to herbicides and brominated flame retardants (BFRs).

In the current study straightness (STR) and linearity (LIN) parameters showed the lowest values in Soummam sperm activated with the water of this polluted River. Surprisingly, these two indicators were enhanced when Soummam sperm is activated with Agrioun non-polluted water. Different authors reported that STR and LIN can be very useful indicators of curvature of the trajectory expressing a progressive motility (Gallego and Asturiano 2019). In this respect, recently, the effect of heavy metals on the motility parameter of zebrafish (*Danio rerio*) showed that progressive motility was the most sensitive parameter of pollution (Kollar et al. 2018). Similarly, in agreement with these reports, the progressive sperm motility significantly decreases in common carp (*Cyprinus carpio*) exposed to mercury (Hg) and Cuprum (Cu) (Kovacik et al. 2018) and in zebrafish (*Danio rerio*) exposed to brominated flame retardants (BFRs) and heavy metals. However, STR and LIN are reported to be significantly higher at concentration of 10 and 100 µg/l of bisphenol-A (BPA) in zebrafish (*Danio rerio*) (Silveira et al. 2019).

Oxidative stress has been defined as an imbalance of oxidants and antioxidants in favour of the oxidants, which potentially leading to cell damages (Pruchniak et al. 2016). Oxidative stress markers are very important tools in sperm analysis, especially in terms of toxicity assessment (Cabrita et al. 2014). In the present study, the oxidative stress was significantly lower in fish living in unpolluted water Agrioun River. In fact, it's well known that the pollution is capable of generating oxidative stress by reducing antioxidant defenses in fish, which may explain in the current study the generation of ROS in the sperm cells of *Barbus callensis* living in Soummam freshwater. The same findings are reported in carp (*Cyprinus*

carpio) (Kovacik et al. 2018) and in sterlet (*Acispenser ruthenus*) (Shaliutina et al. 2017). Such findings could explain the involvement of oxidative stress as an underlying mechanism in sperm motility alternation under water pollution. This could inhibit one or more physiological processes responsible in success of fertilization in fish.

Conclusions

In conclusion, the current investigation showed that *Barbus callensis* sperm motility parameters, particularly spermatozoa concentration, straightness and linearity are good bioindicators of water pollution in addition to sperm cells oxidative stress status.

Declarations

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Figures

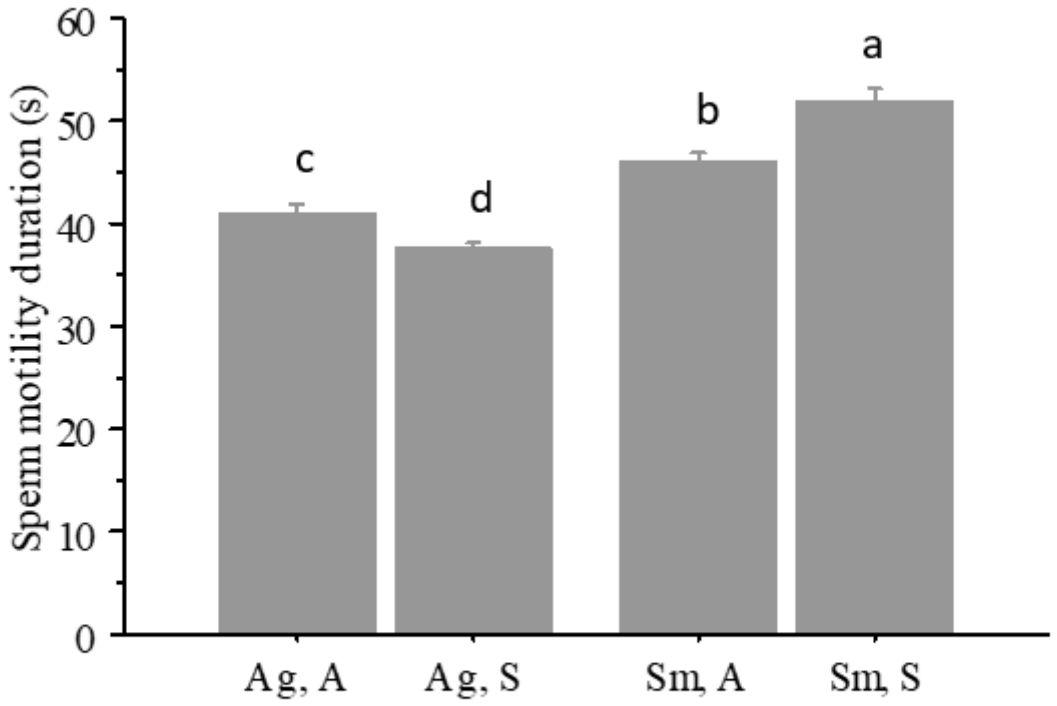

Figure 1

Sperm motility duration (SMD) of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

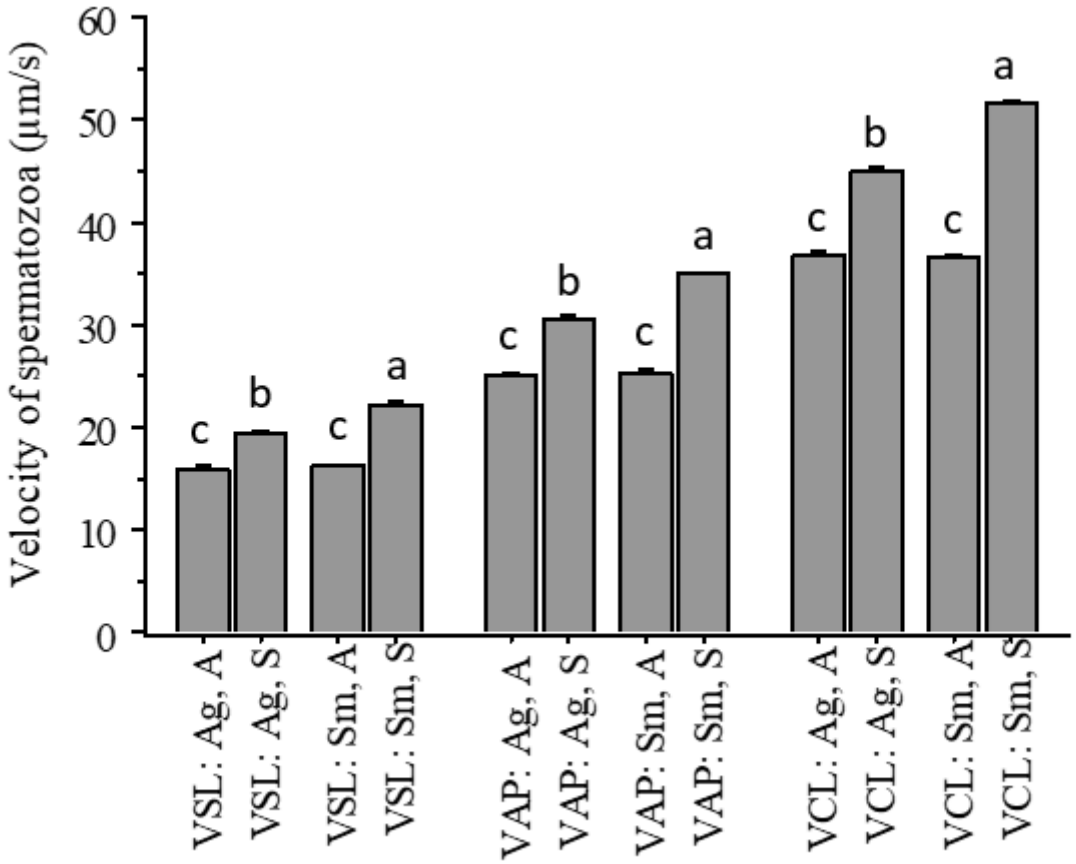

Figure 2

Sperm velocity of *B. callensis* spermatozoa (VSL, VAP and VCL) from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

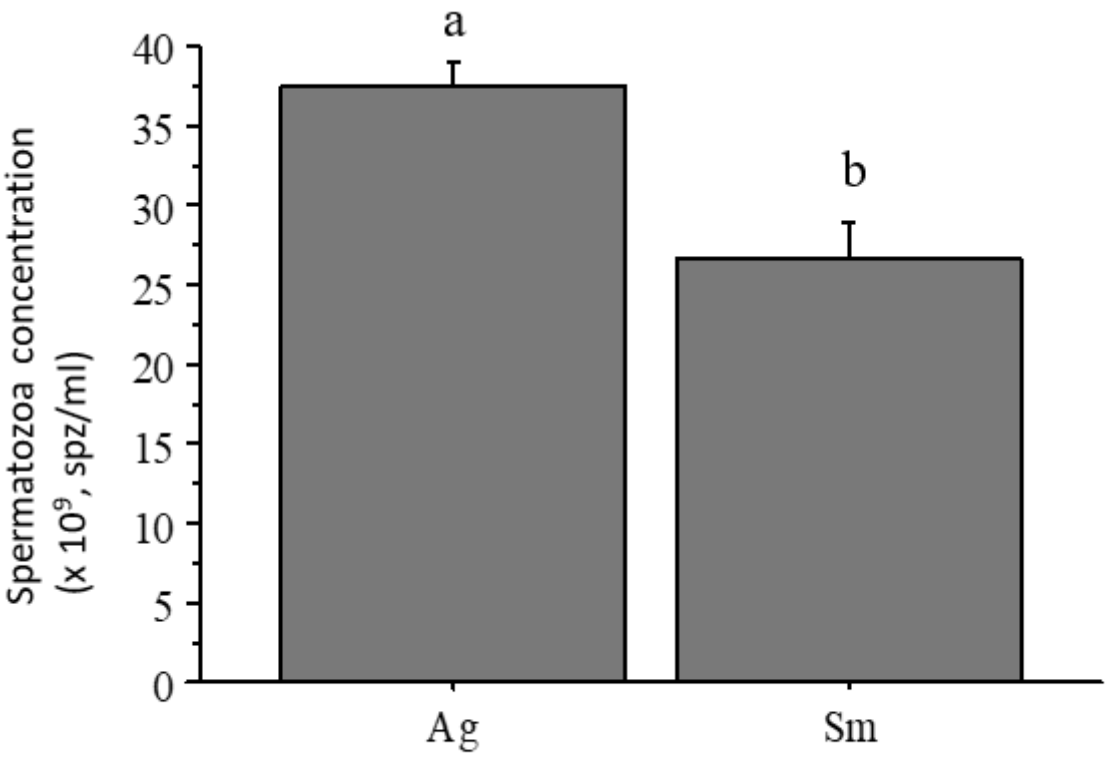

Figure 3

Spermatozoa concentration (SC) of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A) and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

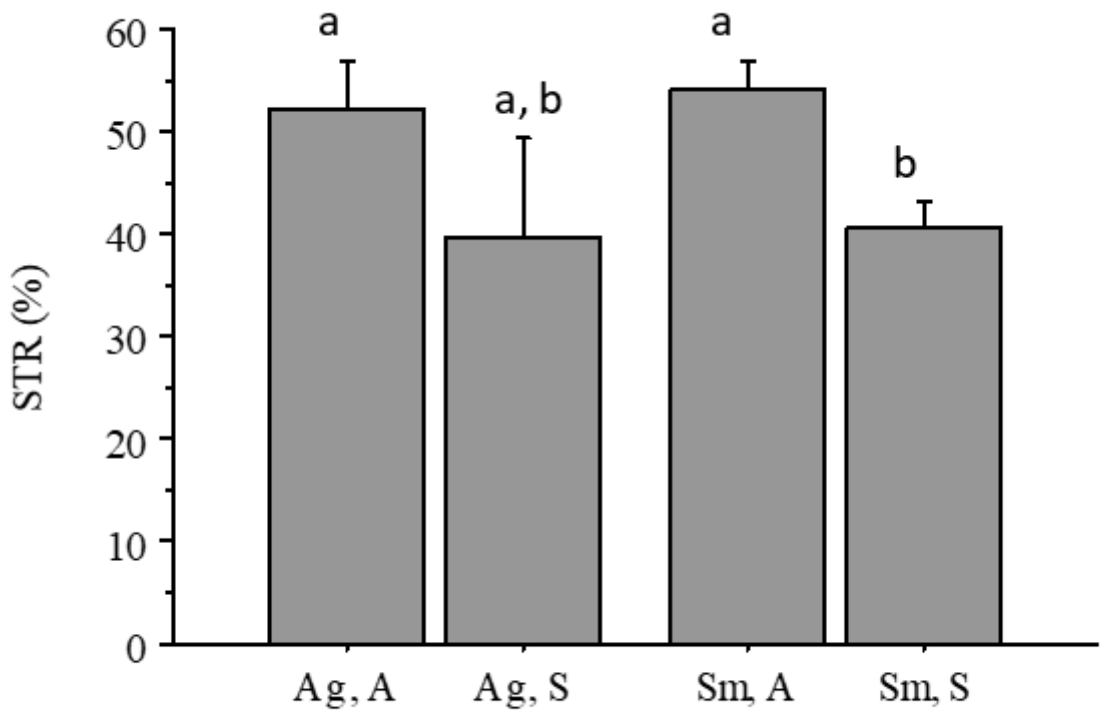

Figure 4

Straightness (STR) of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

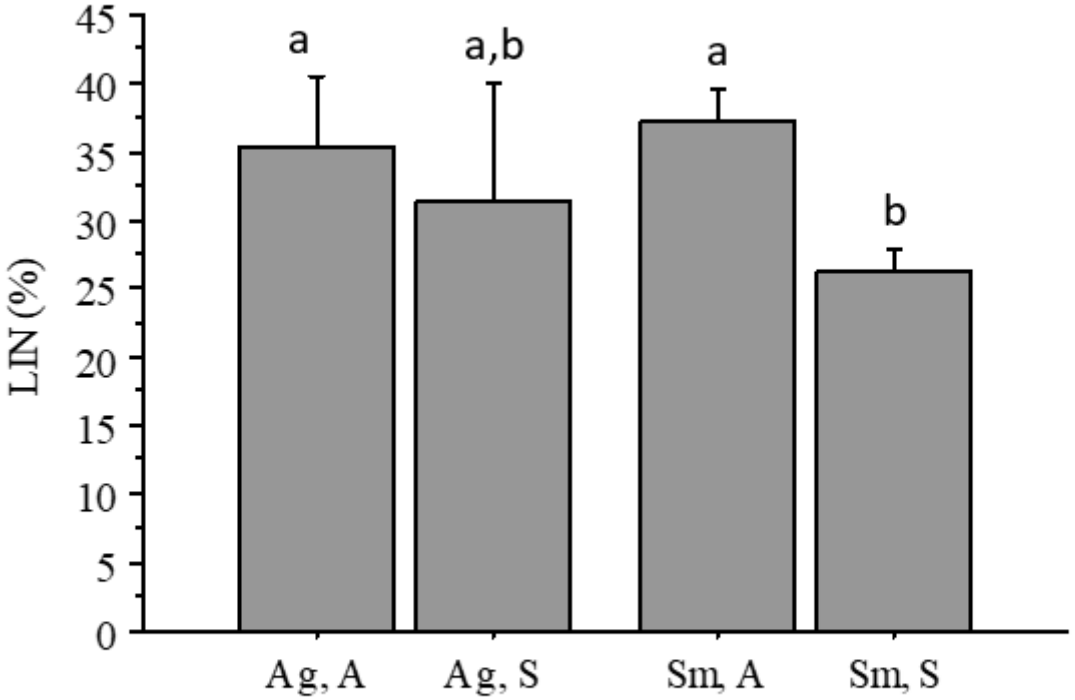

Figure 5

Linearity (LIN) of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

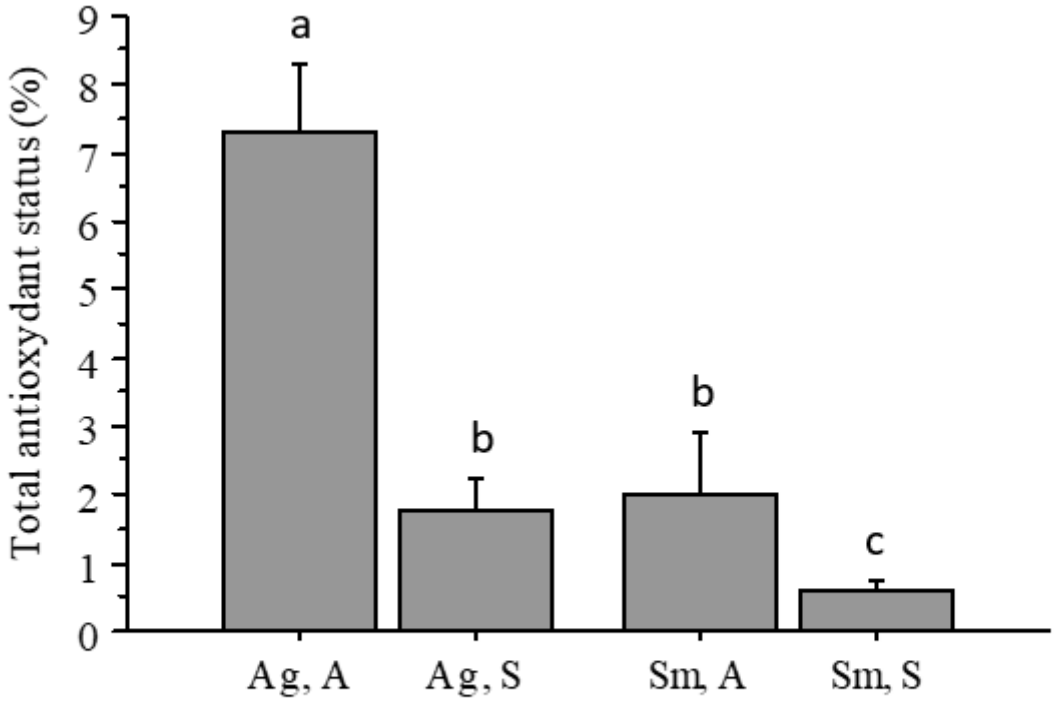

Figure 6

ABTS°+ scavenging activity of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$

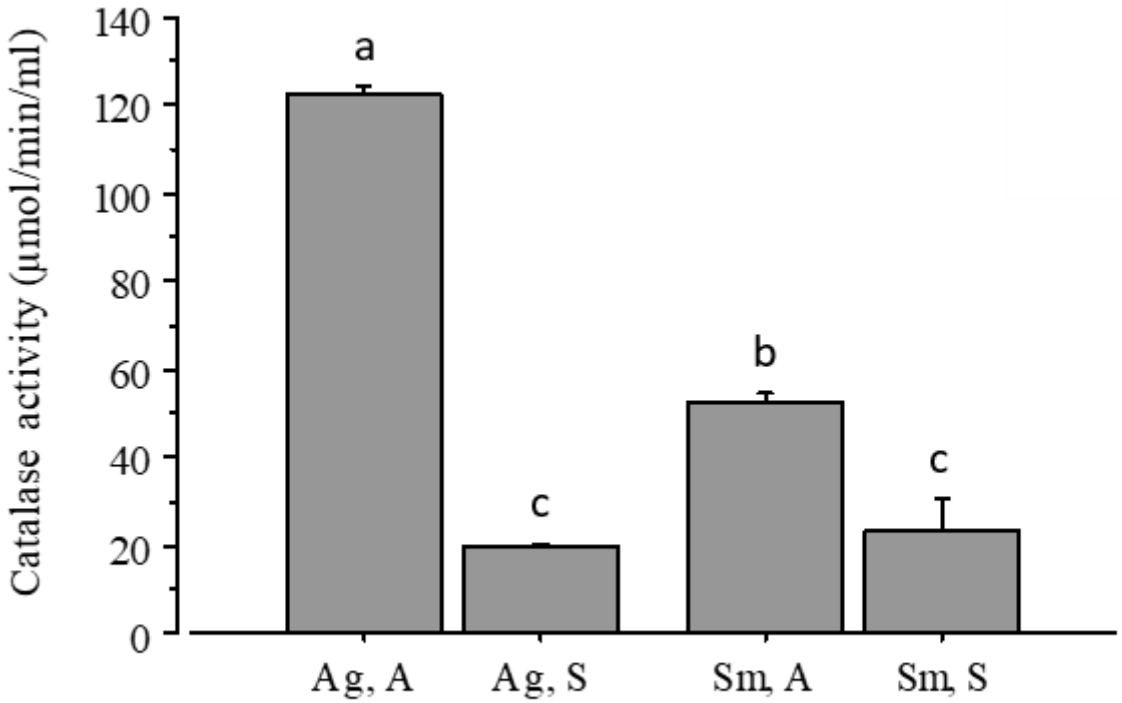

Figure 7

Catalase activity of *B. callensis* spermatozoa from an unpolluted river activated with unpolluted fresh water (Ag, A), spermatozoa from an unpolluted river activated with polluted fresh water (Ag, S), spermatozoa from a polluted river activated with unpolluted fresh water (Sm, A), and spermatozoa from a polluted river activated with polluted fresh water (Sm, S). Values are expressed as mean ± standard deviation (n = 171). Values with different letters are statistically different at $P < 0.05$