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Determination of selenium toxicity to *Oreochromis niloticus* based on hematological parameters

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ABSTRACT. Selenium (Se) is described as an essential micronutrient and participates in different biological functions, as the antioxidant defense systems maintenance and regulation. However, when in high concentrations, Se may cause toxic effects as well as hematological changes in fish. The aim of the present study was to determine the toxicity of selenium in the form of sodium selenate ($Na_2Se^{6+}O_4$) in *Oreochromis niloticus* based on hematological parameters, after exposure to different concentrations (0.01, 0.14 and 1.4 mg Se^{6+} L^{-1}). The erythrocytic and leukocytic series were examined over 14 days at intervals of 0, 3, 5, 7,10 and 14 days. The erythrocytic series showed significant alterations in the first 7 days, including the control group. Neutrophils and monocytes showed variations in the first 3 days at a concentration of 1.40 mgSe^{6+} L^{-1} characterizing an acute response. The total number of leukocytes was different in relation to time zero on all Se concentrations. The thrombocyte count also differed statistically from time zero and control in the first 3 days at 0.14 mgSe^{6+} L^{-1}. These results indicate that different concentrations induce an acute response with diminution of total leukocytes, neutrophilia, monocytosis and thrombocytosis.

Keywords: haematological, water pollution, selenium, toxicity.

Determinação da toxicidade do Selênio por meio de análises hematológicas em *Oreochromis niloticus*

RESUMO. O selênio (Se) é descrito como um micronutriente essencial e participa de diversas funções biológicas. No entanto, quando em concentrações elevadas, o Se pode causar efeitos tóxicos, bem como alterações hematológicas em peixes. O objetivo do presente estudo foi determinar a toxicidade do selênio na forma de Selenato de sódio ($Na_2Se^{6+}O_4$) em *Oreochromis niloticus* com base em parâmetros hematológicos, após a exposição a diferentes concentrações (0,01, 0,14 e 1,4 mg + Se^{6+} L^{-1}). A série eritrocitária e leucocitária foram examinadas por 14 dias, em intervalos de 0, 3, 5, 7,10 e 14 dias. A série eritrocítica mostrou alterações significativas nos primeiros 7 dias, incluindo o grupo controle. Neutrófilos e monócitos apresentaram variações nos primeiros 3 dias na concentração de 1,40 mgSe^{6+} L^{-1}, caracterizando uma resposta aguda. O número total de leucócitos foi diferente em relação ao tempo zero em todas as concentrações de Se. A contagem de trombócitos também diferiram estatisticamente entre o tempo zero e os primeiros 3 dias a 0,14 mgSe^{6+} L^{-1}. O resultados indicam que diferentes concentrações induzem a resposta aguda com diminuição dos leucócitos totais, neutrofilia, monocitose e trombocitose.

Palavras-chave: hematologia, poluição aquática, selênio, toxicidade.

Introduction

Some chemicals elements are of great importance for biological functions, but the concentration is a determinant factor between its benefits and toxicity. Selenium (Se) is considered an essential micro-nutrient due to participation in antioxidant defense system (GUNBY, 1981; STADTMAN, 1980), in protein synthesis (LEMLY, 2002) and in the reduction of toxicity of heavy metals (CHANG, 1979; FRANÇA et al., 2007). Steffens (1989) reported that for fish 0.2 and 0.5 mg kg^{-1} of Selenium is ideal. However, Lemly (2002), Swee et al. (2002) and Hamilton (2004) reported that elevated concentrations can cause disturbances in different levels of biological organization from molecular up to ecological.

The toxicity of selenium can vary according to the different states of oxidation in aquatic environments (FRANÇA et al., 2007; HAMILTON; BUHL, 1990; HILTON et al., 1980; LEMLY, 1993, 2002; NIIMI; LAHAN, 1976; SORENSEN et al., 1984; TAKAYANAGI, 2001). The toxicity of environmental pollutants on aquatic biota can be measured *in situ*, but

there is doubt whether this can potentiate or minimize the biological effects of the contaminant in question. Among the ways of observing such effects on biological systems, minimizing interference from internal and external factors, the utilization of bioassays is a procedure recommended by international environmental organs and helps public policies regarding the environment and public health.

Bioassays with fish have been utilized frequently for evaluating the potential of innumerous substances present in the aquatic environment which can reach humans through the food chain. Besides, fish are important bioindicators of environmental alterations, because they respond sensitively to numerous contaminants at low concentrations and perform different roles in the trophic chain thereby undergoing bioaccumulation or substances from other trophic levels (AL-SABTI; METCALFE, 1995).

Among the ways to determine the effects of physical, chemical and biological agents on fish, hematological analyses represent an important tool for measuring physiological compromise in fish. Studies on alterations of the hematological parameters of fish exposed to pollutants indicate that these analyses are important indicator of the physiological state of the organisms, as well as being able to serve as a basis for the monitoring of environmental pollution and on possible ecological repercussions (FRANÇA et al., 2007; HILTON et al., 1980; ISHIKAWA et al., 2007; LEMLY, 1993, 2002; LOHENR et al., 2001; OLIVEIRA-RIBEIRO et al., 2000; RANZANI-PAIVA et al., 1997; SORENSEN et al., 1984).

Oreochromis niloticus, due to its rusticity and broad knowledge of techniques for its cultivation and breeding in captivity, makes up a considerable portion of the world production of fish. Elucidating the effects of contaminants on physiological aspects and factors that can enhance return, is part of the productive chain, mainly those that can be measured at the lower level of biological organization when organisms are exposed to different times and/or concentrations of stressor agents. Thus, the aim of the present study was to determine alterations in the hematological parameters of tilapia, *O. niloticus*, exposed to different concentrations of selenium in the form of sodium selenate ($Na_2Se^{6+}O_4$) using a chronic toxicity test.

Material and methods

Juveniles of *O. niloticus*, with a mean weight of 30.66 ± 4.67g and mean length of 12.11 ± 0.86 cm were acquired from commercial fish farm and acclimated to laboratory conditions for 7 days in 250-L tanks with dechlorinated water, constant aeration and feeding with commercial extruded ration.

The toxicity tests were conducted as described in the Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WPCF, 2005) in 40L aquariums available in three replicates, containing three different selenium concentrations and negative control.

The Se^{6+} concentrations of 0.01, 0.14 and 1.40 mg L^{-1} were prepared from sodium selenate ($Na_2Se^{6+}O_4$, Synth®), prepared at a proportion of 1.666 g in 500 mL of MILLI-Q water, resulting in a stock solution of 1000 mg Se^{6+} L^{-1}, which was later diluted to the above-mentioned concentrations. The experiment was conducted for 14 days at a density of 16 fish per aquarium. Samplings consisted of two individuals per treatment for each replicate, totaling six individuals per treatment at times 0, 3, 7, 10 and 14 days.

After anesthesia with benzocaine, blood was drawn by caudal puncture, with the help of a needle and syringe previously heparinized. The blood specimens were assayed for: number of erythrocytes (RBC), counted in a Neubauer chamber; hematocrit (Ht) by the microhematocrit technique; and hemoglobin level (Hb) by the cyanomethemoglobin method. After these procedures, the following RBC indices were calculated: MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration). Smears were made using the same blood aliquots, and the slides were later stained with May-Grunwald-Giemsa according to Rosenfeld (1947), which were utilized for total leukocyte number (WBC) and differential count for each type of leukocyte (lymphocytes, neutrophils, basophils, eosinophils and monocytes) and total thrombocytes by the indirect method according to Hrube and Smith (1998).

In order to assure that the probable toxic effects observed were attributed to sodium selenate, the following physical and chemical variables of the water were measured: temperature (°C), dissolved oxyen (mg L^{-1}), pH, conductivity (µS cm^{-1}), total ammonia (NH_4^+, mg L^{-1}) and hardness as $CaCO_3$ (mg L^{-1}) (APHA/AWWA/WPCF, 2005). Non-ionized ammonia (NH_3, mg L^{-1}) was calculated based on pH and temperature according to Bower and Bidwell (1978).

The results obtained were evaluated by ANOVA and Tukey's test. Alterations were considered significant when $p < 0.05$ (ZAR, 1996).

Results and discussion

The means of the physical and chemical variables of the water are presented in Table 1 and

demonstrate that there were no significant differences among the treatments.

Table 2 shows that the number of erythrocytes (RBC) was affected at all concentrations of Se in relation to control and time zero. There was a significant reduction ($p < 0.05$) over time.

The mean values of Hb had no significant alterations when comparisons were made between the control, time zero and the Se concentrations of 0.01 and 0.14 mg L^{-1}, as well as in relation to time. However, at the concentration of 1.40 mg Se^{6+} L^{-1} on day 14 there was a reduction in Hb where there was significant difference ($p < 0.05$) in relation to time zero, control and the other Se concentrations.

The RBC indices showed significant variations in relation to control, Se concentrations and time. At concentrations of 0.14 and 1.40 mg Se^{6+} L^{-1} it was possible to see a significant increase in MCV up to day 7 in relation to time zero and control. Afterward, alterations were observed, albeit not significant. At a concentration of 1.40 mg L^{-1}, a significant decline occurred between the 7th and 10th days.

At Se concentrations of 0.01 and 0.14 mg Se^{6+} L^{-1} and in the control, there was an increase in MCHC in the first three days, but with no significance differences, which was followed by a decline. At a concentration of 1.40 mg Se^{6+} L^{-1}, this variable showed a significant increase on day 7 in relation to time zero, control and the other Se concentrations. The effects were only observed at a concentration of 1.40 mg Se^{6+} L^{-1} between the 7th and 14th day, due to a sudden elevation in MCHC in this period.

The number of thrombocytes showed a significant difference in comparison with control and time zero on days 3, 10 and 14 at concentrations of 0.14 and 1.40 mg Se^{6+} L^{-1}. Over time, no significant alteration was observed with any other Se concentrations.

There was a significant reduction in WBC over time in relation to time zero at a concentration of 0.01 mg Se^{6+} L^{-1}, up to the 7th day followed by a subtle elevation up to day 14. In a comparison among the Se concentrations and control on the 3rd, 7th and 14th days, no significant differences were found. Only on day 10 there was an elevation in the number of leukocytes with Se concentrations of 0.14 mg Se^{6+} L^{-1} and 1.40 mg Se^{6+} L^{-1} with a significant difference in relation to control and the other Se concentrations.

Table 1. Means of physical and chemical variables of water in the chronic toxicity test of sodium selenate (Se^{6+}) in *O. niloticus*.

mg Se^{6+} L^{-1}	Temperature (°C)	Dissolved Oxygen (mg L^{-1})	pH	Conductivity (µS cm^{-1})	Amonia (mg L^{-1})	Unionized amonia (NH_3) (mg L^{-1})	Hardness (mg $CaCO_3$ L^{-1})
Control	24.11 ± 1.36[a]	6.04 ± 0.61[a]	7.45 ± 0.26[a]	139.37 ± 37.27[a]	7.93 ± 0.21[a]	0.1146[a]	22.21 ± 1.13[a]
0.01	23.7 ± 1.57[a]	6.41 ± 0.70[a]	7.57 ± 0.21[a]	122.51 ± 32.40[a]	6.97 ± 1.08[a]	0.1283[a]	20.25 ± 2.99[a]
0.14	23.75 ± 1.44[a]	6.29 ± 0.81[a]	7.63 ± 0.24[a]	142.75 ± 48.43[a]	7.5 ± 1.21[a]	0.1586[a]	20.25 ± 2.99[a]
1.40	24.29 ± 1.57[a]	6.36 ± 0.83[a]	7.66 ± 0.22[a]	125.14 ± 33.85[a]	7.5 ± 0.4[a]	0.1765[a]	21.56 ± 1.9[a]

*ab = medium followed the same letter do not differ statistically.

Table 2. Red blood serie of *Oreochormis niloticus* exposed to selenium.

Groups	days	Ht (%)	Hb (g dL^{-1})	Er (10^4 mL^{-1})	VCM (fL)	CHCM (%)
Zero						
x ± SD	0	27.94 ± 3.99[a]	8.25 ± 1.09[a]	237.75 ± 28.53[a]	117.66 ± 11.34[a]	29.63 ± 2.31[a]
control						
x ± SD	3	24.08 ± 3.84[ab]	7.77 ± 1.24[a]	227.17 ± 34.51[a]	106.10 ± 6.24[a]	32.30 ± 1.45[a]
x ± SD	7	21.75 ± 2.48[ab]	6.46 ± 1.35[a]	143.08 ± 25.23[a]	153.87 ± 14.72[a]	29.50 ± 4.22[a]
x ± SD	10	23.58 ± 3.12[ab]	7.21 ± 1.28[a]	136.17 ± 18.85[a]	173.87 ± 17.67[a]	30.47 ± 2.18[a]
x ± SD	14	22.33 ± 1.40[ab]	6.90 ± 0.87[a]	142.75 ± 22.24[a]	159.04 ± 21.78[a]	30.84 ± 2.11[a]
0.01 mg Se^{6+} L^{-1}						
x ± SD	3	22.75 ± 2.60[b]	8.11 ± 0.49[a]	169.92 ± 47.24 [ab]	138.65 ± 22.58[ab]	35.93 ± 3.59[a]
x ± SD	7	23.17 ± 4.32[ab]	7.21 ± 0.87[a]	153.00 ± 26.27[b]	157.07 ± 48.61[ab]	31.71 ± 4.83[a]
x ± SD	10	25.42 ± 3.63[ab]	7.78 ± 1.37[a]	170.58 ± 37.23[b]	152.89 ± 27.05[ab]	31.03 ± 6.66[a]
x ± SD	14	24.50 ± 3.16[ab]	6.98 ± 1.50[a]	148.50 ± 28.06[ab]	168.47 ± 30.04[ab]	28.34 ± 3.67[a]
0.14 mg Se^{6+} L^{-1}						
x ± SD	3	25.67 ± 3.76[ab]	8.19 ± 1.29[a]	194.33 ± 56.66[ab]	139.22 ± 31.98[b]	31.93 ± 2.37[a]
x ± SD	7	23.50 ± 2.65[ab]	7.40 ± 1.09[a]	186.25 ± 45.87[ab]	134.39 ± 45.24[b]	31.85 ± 6.33[a]
x ± SD	10	25.42 ± 4.79[ab]	7.72 ± 1.45[a]	179.50 ± 34.41[ab]	144.15 ± 30.01[ab]	30.37 ± 1.76[a]
x ± SD	14	22.50 ± 2.72[c]	6.53 ± 0.74[a]	155.67 ± 24.67[b]	145.48 ± 11.53[ab]	29.11 ± 1.98[a]
1.40 mg Se^{6+} L^{-1}						
x ± SD	3	26.33 ± 3.37[b]	7.86 ± 0.76[a]	177.92 ± 48.31[ab]	157.22 ± 43.61[b]	29.98 ± 1.93[a]
x ± SD	7	23.50 ± 2.93[ab]	7.02 ± 1.03[a]	144.17 ± 37.42[b]	168.63 ± 28.70[b]	30.00 ± 4.02[a]
x ± SD	10	23.17 ± 2.73[ab]	7.74 ± 1.00[a]	175.33 ± 19.60[ab]	132.40 ± 9.42[a]	33.38 ± 1.93[c]
x ± SD	14	20.00 ± 4.67[ab]	5.94 ± 1.68[b]	119.92 ± 19.20[ab]	165.68 ± 21.59[ab]	29.41 ± 2.88[d]

*ab = medium followed the same letter do not differ statistically.

With regard to leukocytes, neutrophil counts did not show a significant difference in relation to the different concentrations. However, compared to time zero, there was a significant reduction on the 3rd and 10th days at a concentration of 0.01 mg L^{-1}. At a concentration of 0.14 mg L^{-1}, the effect was only observed on the 7th day, and from that time on, the response was similar to that observed with 0.01 mg Se^{6+} L^{-1}. At a concentration of 1.40 mg L^{-1}, there was a difference between time zero and day 3, which showed an increase, and on day 14 day, there was a marked fall in neutrophil numbers in relation to time zero.

Monocytes displayed a significant increase in relation to time zero on the 3rd day at Se concentrations of 0.14 and 1.40 mg Se^{6+} L^{-1}, while control and the concentration of 0.01 mg Se^{6+} L^{-1} showed a significant reduction. In the other samples, with time and among the different concentrations, there were no significant differences.

Lymphocytes showed a significant increase in relation to time zero and control only on the 10th day for all Se concentrations.

Eosinophils and basophils were not found.

In the present study, the hematological responses observed were caused by the action of sodium selenate, since the water did not show significant alterations over time in their physical and chemical characteristics. In addition, the values obtained are within the range recommended by Boyd (1982), as being safe for the rearing of fish without there being alterations in physiological integrity and in production (Table 1).

It is unlikely that ionized ammonia levels, even though elevated, had any influence on the hematological parameters determined in the present study. According to Noga (1995), they are values considered to be below the levels that are toxic to fish.

Hargreaves and Kucuk (2001) observed that ammonia in concentrations below 0.28 mg L^{-1} did not lead to toxic effects in *O. aureus*. In addition, Xu et al. (2005) did not observe any behavioral alterations in *O. niloticus* exposed to 0.13 mg L^{-1} of ammonia. The higher values found with these two concentrations can be linked to the probable alterations in gill activities, since this organ acts in the excretion of nitrogenous compounds, but the higher pH and temperature were the determinant factors that could better explain such concentrations of the non-ionized fraction.

The erythrocytic series showed numerous alterations that corroborate the results of similar studies with metals (FRANÇA et al., 2007; ISHIKAWA et al., 2007; JOHANSSON-SJOBECK; LARSON, 1978; OLIVEIRA-RIBEIRO et al., 2000; O'NEIL, 1981; SORENSEN; BAUER, 1984; SORENSEN et al., 1984; THOMAS, 1990), but they show particular characteristics at each concentration.

Os values found in Ht, Er, Hb corroborate findings by Sorensen and Bauer (1984) and Sorensen et al. (1984) in *Lepomis cyanellus* in which they observed a reduction in these parameters in fish exposed to different concentrations of selenium. In the present study, this had trends with alterations observed in MCV which showed higher values with an inverse relation to Ht, Er and Hb.

It is likely that the increase in MCV was due to the release of erythroblasts that are of larger size and do not necessarily contain large amounts of Hb. Further, with this release of erythroblasts, there was no increase in Er and Ht, which indicates a probable hemolytic effect of sodium selenate and a compensation by the release of immature cells.

In relation to Hb and Er, Hilton et al. (1980) and Sorensen (1991) reported that the disproportionality between these parameters indicates that selenium influences Hb formation much more than erythropoiesis. These responses can be explained based on the findings of Thomas (1990) who reported the effects of metals involved in erythropoiesis and biosynthesis of the heme group. In relation to the decline in Hb, Barbosa et al. (1998) and Jacques-Silva et al. (2001) observed that selenium inhibits delta-aminolevulinic acid dehyratase (ALA-D) and interferes with the formation of hemoglobin (BHAGAVAN, 1992). Thus, it is possible to state that based on the above-mentioned studies, that sodium selenate induced the same responses during the first 7 days of exposure. Therefore, it is necessary to better elucidate the mechanism of toxicity of sodium selenate on the biosynthesis of hemoglobin, since the present study demonstrated this toxicity.

In relation to total number of leukocytes, significant leukopenia was seen at the lower concentration.

Basophils and eosinophils were not found in the peripheral blood of fish exposed to different concentrations, nor in the control group. According to Ranzani-Paiva et al. (2005), the percentage of these cells in *O. niloticus* varies around 0.3 and 0.03%, respectively, being difficult to visualize. However, it was not possible to detect any effects on these cells.

Neutrophils showed a constant decline, Thus, other bioassays with metals demonstrate that the first 3 days are typically critical to the exposure to metals showing alterations in this type of leukocyte (MIKRYAKOV; LAPIROVA, 1997). At the higher

Se^{6+} concentration of 1.40 mg L^{-1}, an increase was observed in the first days, this result being similar to that of Ranzani-Paiva et al. (1997) in *Prochilodus lineatus* exposed to Trichlorfon for 78h. Mahajan and Dheer (1979) and Ranzani-Paiva et al. (1997, 2005) suggested that the neutrophils are the most important leukocytes in the peripheral blood of fish and that they show a high sensitivity to modifications in the environment. According to Sorensen (1991), the diminution of the number of neutrophils can possibly indicate a disruption of phagocytic capacity and consequently a reduction in resistance of fish to pathogens. At the highest Se^{6+} concentration of 1.40 mg L^{-1}, an immediate increase in these cells was observed, and with continuous exposure, there was a gradual decline, indicating that these leukocytes could migrate to sites possibly damaged by a toxic effect, or the leukopoietic centers could be lesioned, as observed earlier by Lemly (2002) and Sorensen et al. (1982, 1984) impeding the release of these cells. According to Barton and Zitzow (1995), the increase in neutrophil count is noted as a typical response resulting from the presence of a stressor agent, in this case sodium selenate. With respect to reduction over time, O'Neil (1981) reported that exposure to metals can block the active site of antibodies and the division of immunocompetent cells. In the present study, this fact although measured indirectly by the decrease in the total number of leukocytes – could be observed only after the 7th day.

Monocytes have the capacity to migrate to lesioned foci, and therefore it is likely that at the highest selenium concentrations this could have occurred due to the fact that acute exposure to high doses of selenium causes lesions in some tissues.

With respect to the lymphocytes, an increase was observed on the 10th day at a concentration of 0.01 mg L^{-1}, which have been attributed to the chronic exposure, since these cells are involved in inflammatory processes (LAMAS et al., 1994). Lymphopenia is a typical signal of stress from environmental stressors including metals (JOHANSSON-SJOBECK; LARSON, 1978). The results of the present study diverge from those reported by Lemly (1993) who found increased lymphocyte counts. Lymphopenia correlated with leukopenia, which denotes the greater percentage of these cells in the bloodstream of *O. niloticus* (TAVARES-DIAS; MORAES, 2004). In addition, and together with the alterations observed in the neutrophils, monocytes and thrombocytes, it is characteristic of acute stress.

The results presented with respect to the significant alterations in the monocytes and neutrophils in the first 3 days corroborate the findings of Mikryakov and Lapirova (1997) and França et al. (2007) who observed an increased number of these cells in the first days of exposure to selenium.

Alterations in the total number of thrombocytes were found by Lemly (2002) and Sorensen et al. (1984), in fish from environments contaminated with selenium. The thrombocytes of fish have structures similar to the platelets of mammals (MORROW; PULSFORD, 1980), where they are also considered responsible for the process of blood coagulation (CASSILAS; SMITH, 1977) and can even act in defense processes (MATUSHIMA; MARIANO, 1996). Correlating the increased numbers

Table 3. Thrombocytes and leukocytes of *Oreochormis niloticus* exposed to selenium.

Zero	Days	Lc mm^{-3}	Tr mm^{-3}	Nt mm^{-3}	Mn mm^{-3}	Lf mm^{-3}
x ± SD	0	49143.33 ± 334.65^{a}	9615.00 ± 138.70^{a}	6404.40 ± 33.73^{a}	1948.50 ± 12.36^{a}	40790.50 ± 325.56^{a}
control						
x ± SD	3	30215.42 ± 148.72^{ab}	11247.92 ± 195.65^{a}	1370.11 ± 15.77^{ab}	1030.82 ± 7.87^{ab}	27814.48 ± 145.50^{a}
x ± SD	7	26417.92 ± 471.29^{ab}	8082.92 ± 84.68^{a}	2635.60 ± 68.56^{ab}	2007.04 ± 46.97^{ab}	21775.28 ± 362.48^{a}
x ± SD	10	19300.83 ± 99.23^{ab}	2605.42 ± 45.75^{a}	637.74 ± 5.57^{ab}	446.89 ± 1.35^{ab}	18216.21 ± 96.57^{a}
x ± SD	14	27950.83 ± 256.36^{ab}	6512.92 ± 52.98^{a}	1220.68 ± 20.30^{ab}	925.31 ± 12.64^{ab}	25790.31 ± 250.54^{a}
0.01 mg Se^{6+} L^{-1}						
x ± SD	3	24818.33a ± 268.65^{b}	8563.75 ± 147.35^{ab}	1415.70 ± 15.22^{b}	873.61 ± 10.64^{b}	22517.60 ± 249.71^{a}
x ± SD	7	15323.75 ± 138.32^{b}	9782.08 ± 105.19^{ab}	1386.15 ± 9.66^{ab}	786.39 ± 2.92^{ab}	13147.85 ± 141.75^{a}
x ± SD	10	26701.67 ± 150.93^{ab}	5566.67 ± 103.56^{ab}	1867.93 ± 18.59^{b}	1719.58 ± 23.35^{ab}	23108.95 ± 158.62^{b}
x ± SD	14	24557.08 ± 251.15^{ab}	4083.33 ± 62.14^{ab}	1325.96 ± 13.87^{ab}	893.09 ± 9.77^{ab}	22338.04 ± 236.35^{a}
0.14 mg Se^{6+} L^{-1}						
x ± SD	3	37731.67 ± 410.69^{ab}	16133.33 ± 158.80^{b}	4436.06 ± 63.84^{ab}	2963.52 ± 31.56b	30332.09 ± 349.65^{a}
x ± SD	7	31001.25 ± 248.96^{ab}	16624.17 ± 60.30^{ab}	1322.98 ± 11.85^{ab}	1324.72 ± 12.53ab	28353.55 ± 241.36^{a}
x ± SD	10	44151.25 ± 146.06^{c}	8315.42 ± 118.20^{b}	2007.20 ± 21.99^{ab}	1808.90 ± 19.93ab	40335.15 ± 137.22^{b}
x ± SD	14	33577.92 ± 122.20^{ab}	14248.33 ± 121.57^{b}	1658.03 ± 21.15^{ab}	1085.31 ± 6.16^{ab}	30834.58 ± 112.64^{a}
1.40 mg Se^{6+} L^{-1}						
x ± SD	3	47081.25 ± 384.77^{ab}	14069.58b ± 134.67^{b}	7110.84 ± 116.77^{b}	2984.86 ± 28.44^{b}	36985.54 ± 298.62^{a}
x ± SD	7	34302.92 ± 217.14^{ab}	7580.83 ± 69.92^{ab}	1553.71 ± 19.87^{ab}	1160.96 ± 6.64^{ab}	31588.24 ± 197.16^{a}
x ± SD	10	37137.92 ± 229.03^{c}	8698.75 ±+ 164.76^{b}	1518.60 ± 11.64^{ab}	1293.88 ± 10.33^{ab}	34325.43 ± 215.96^{b}
x ± SD	14	23731.25 ± 165.44^{ab}	3914.17 ± 47.93^{b}	882.13 ± 15.07^{b}	814.39 ± 10.48^{ab}	22034.74 ± 145.4^{2a}

*ab = medium followed the same letter do not differ statistically.

of these cells with the decline in Er and Ht as indicators of possible hemorrhagic foci – as observed previously by Mazon et al. (2002) – and considering their similar function compared to mammalian platelets, the increase in these cells would explain the likely internal lesions (kidney and liver) or external ones (gills), which would need their counts. In addition, as already noted by Matushima and Mariano (1996) on the function of thrombocytes in defense, it is probable that selenate acts a foreign substance to be phagocytized, and other defense cells such as monocytes and neutrophils would be involved with thrombocytes, more aggressively than the lymphocytes.

Conclusion

In the present study, it was evident that exposure to sodium selenate caused alterations in the hematological parameters of *O. niloticus*. Even though significant differences were not always found in the majority of the samplings at the different concentrations, there were alterations in the blood elements within the first 7 days. This indicates that acute effects such as alteration in RBC indices and chronic effects on leukocytes can be indicative of stress from exposure to sodium selenate.

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