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Effects of major and trace elements from the El Kahfa ring complex on fish: Geological, physicochemical, and biological approaches

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The alkaline rocks are known for enriching rare lithophilic elements, including lithium, uranium, and tin, which negatively impact aquatic life. This study offers an intensive investigation of the influence of alkaline rocks on *Nile Tilapia* (*Oreochromis niloticus*). The variation in blood profile, the induction of antioxidant enzymes, morphological erythrocyte, and histological structure have been conducted for the fish after 15 days of exposure to alkaline rocks powder with a dose of 100 µg/L. As a result, there was a pronounced decrease in blood profiles, such as platelets and white blood cell counts. There was a failure in the liver and kidney functions. Moreover, it shows an increase in superoxide dismutase (SOD) and catalase (CAT) activities as antioxidant biomarkers. Also, exposure to alkaline rocks induced DNA mutation and erythrocyte distortion. We concluded that the bulk alkaline rocks induced changes in the hemato-biochemical and antioxidant parameters of *Nile tilapia*. Additionally, exposure to bulk alkaline rock compounds also caused poikilocytosis and nuclear abnormalities of RBCs. This draws our attention to the seriousness of climatic changes, the erosion of rocks, and their access to water.

KEYWORDS

Alkaline rocks, SOD, DNA, antioxidant, blood profile

1 Introduction

Alkaline rocks are characterized by the presence of feldspathoids, alkaline pyroxenes, amphiboles, or all of them combined. One of the main reasons that has attracted petrologists to conduct intensive studies of alkaline rocks is their enrichment in rare lithophilic elements, such as Be, Nb, Ta, Zr, Hf, Ga, Li, Rb, Th, U, and rare earth elements (REE). However, alkaline rocks are of minimal distribution around the world. In North

America, alkaline rocks cover an area of less than 0.05% of the total area of igneous rocks. In Europe, the ratio is likely to be smaller than 1% (Harangi et al., 2003). In Egypt, alkaline rocks are of relatively limited distribution, particularly in the southeastern desert. They are represented mainly by ring complexes, some dykes, plugs, volcanic flows, and, comparatively, small alkaline granitic, syenitic bodies, or both. The detailed geology, petrology, geochemistry, and geochronology of the El Kahfa ring complex have been treated by several authors (El Ramly et al., 1969; Serencsits et al., 1981; El Ramly and Hussein 1985; Bishady et al., 2016; Hegazy et al., 2016). El Ramly et al. (1969) inferred that the El Kahfa ring complex intruded into metavolcanic and epidiorite rocks. The El Kahfa ring complex comprises an incomplete ring and a small central stock. The incomplete ring comprises inner and outer rings and consists of alkaline syenites, quartz-bearing syenites, and alkali gabbro. The central stock is formed from small bodies of volcanic rocks and mainly of alkali gabbro, which is cut by small bodies of alkaline syenite that grade into nepheline syenite. These rocks are enriched in the Lithophile Elements (LILE) (Rb and Ba) and the high-field strength element (HFSE) (Zr, Hf, Ta, and Nb) (Pfander et al., 2007).

The importance of REEs in a wide range of technologies arises from their natural availability. One of the most common sources of REE is its dispersion from indigenous rocks, which may cause water contamination. Thus, contamination may contribute to their release into surrounding ecosystems and aquatic organisms (Malhotra et al., 2020). Rock deterioration along the series of mountains of the Red Sea region can increase the exposure of terrestrial and aquatic organisms to high levels of REEs. There is a shortage in studying the effects of REEs on aquatic biota, given the limited number of reported studies on the toxicity of REEs on the aquatic environment and organisms. Hence, the impact of REE toxicity on aquatic organisms should be thoroughly discussed, considering growth inhibition, embryotoxicity, cytogenetic effects, and organ-specific toxicity. Aquatic biota was reported as a significant models for studying the toxic influence of REEs and improving their impacts on human health (Malhotra et al., 2020).

Moreover, there are some studies in which REEs have been shown to have potential cytotoxicity and genotoxicity properties (Franchi et al., 2015) on animals models, such as zebrafish (He et al., 2016) and rats (Pagano et al., 2015; Kim et al., 2017). *Nile tilapia* (*Oreochromis niloticus*) is considered a standard aquatic animal model for examining toxicity, especially in acute toxicity studies. Juveniles of tilapia are established as one of the standard models used for toxicity investigation due to their high sensitivity to low-level environmental pollutants (Lan et al., 2021). Different blood cell indices, including hemotoxic and biochemical indices, are used as significant biomarkers of the systemic response to environmental stress in fishes (Mekawy et al., 2011; Abou Khalil et al., 2017; Sayed and Soliman 2018). These indices were

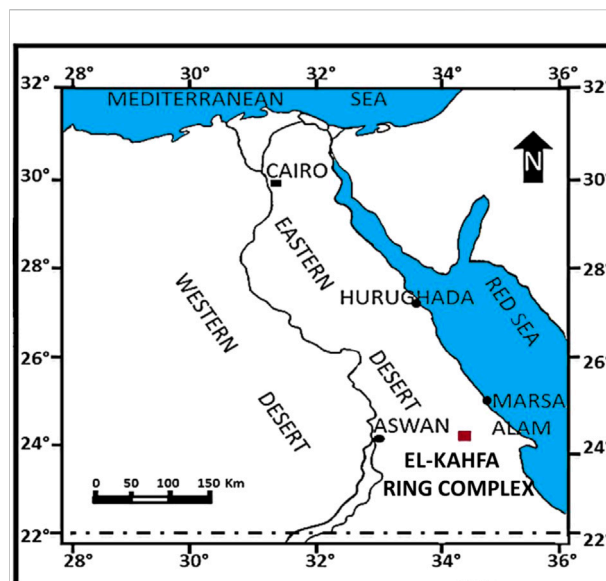


FIGURE 1
Map of the location of the Gabal El Kahfa ring complex.

selected because they are the first route for pollutants and the most sensitive tissue to detect side effects and pathology. Besides nuclear damage, apoptosis, and micronuclei abnormalities, fish erythrocyte distortion was considered in toxicological studies as a sign of pollution (Mekawy et al., 2011; Sayed et al., 2016a; Sayed et al., 2019; Talapatra and Banerjee, 2005). Based on this point of view, this work aims to explore the effects of igneous rock powder on Nile tilapia juveniles using hematobiochemical parameters, erythrocyte alterations, and antioxidant biomarkers as endpoints.

2 Materials and methods

2.1 Sample collection

Alkaline rocks [alkaline syenites (samples I (high Pb and U) and II (low Pb and U)), trachyte (sample III), and quartz-syenites (sample IV) were collected from the Gabal El Kahfa ring complex, which is located in the Southeastern Desert of Egypt, approximately 100 km from the Red Sea coast. It is bounded by longitudes 34°36' to 34°40' E and latitudes 24°04' to 24°10' N (Figure 1).

2.2 Physicochemical analysis

2.2.1 X-ray fluorescence

Major element chemical analyses of four representative rock samples of alkaline syenites and trachyte from the El Kahfa ring complex were done using the XRF technique and ICP-MS.

2.2.2 X-ray diffractometer

An X-ray diffractometer (XRD, Philips PW 2103, Netherland, $\text{CuK}\alpha = 1.54056 \text{ \AA}$ as the radiation source) was employed to analyze the crystal structures and phase compositions of the samples. Diffraction patterns were investigated within the 2θ range of $4\text{--}80^\circ$.

2.2.3 Fourier transform infrared

Infrared spectra were recorded using a Nicolet FTIR spectrophotometer (model 6700) in the wavenumber range of $4,000\text{--}400 \text{ cm}^{-1}$. Measurements were carried out by pelletizing the samples with KBr.

2.2.4 Scanning electron microscope

The morphology and microstructures of the samples were evaluated using a scan electron microscope (SEM, EVO 15, ZEISS, Germany).

2.3 Fish

Noncontaminated young juveniles of the *N. tilapia* (*O. niloticus*) fish that were free of external parasites were collected from the Fish Biology and Pollution Laboratory at Assiut University, Egypt. The young juveniles with an average weight of about 3 g were kept in water tanks under conductivity of $260.3 \mu\text{S/cm}$, pH of 7.4, and dissolved oxygen of 6.8 mg/L , 25.5°C . Moreover, fish were fed 3% of their body weight on commercial diets daily with 12:12 light as a photoperiod for 2 weeks.

2.4 Experimental design

The fish were divided into four groups. Each group contained six fish in each replicate (three experimental replicates). Control group was feed normal in clean water, the 2nd group exposed to $100 \mu\text{g/L}$ from alkaline syenites [sample I (high Pb and U)], 3rd group exposed to $100 \mu\text{g/L}$ from alkaline syenites [sample II (low Pb and U)], 4th group exposed to $100 \mu\text{g/L}$ from trachyte (sample III), and 5th group exposed to $100 \mu\text{g/L}$ from quartz-syenites (sample IV) for 15 days. Every day, half of the capacity of the tanks was replaced with fresh-dosed water. The dose was selected according to the toxic dose studied by Joonas et al. (2017).

2.5 Blood sample collection

At the end of the exposure, fish from each group were randomly selected and anesthetized using ice to lessen stress (Wilson et al., 2009). Then, blood samples were collected from the caudal veins into heparinized and non-heparinized tubes for hematobiochemical parameter measurements. Likewise, blood

smears on clean glass slides were prepared (triplicate slides from each fish/nine slides from each group).

2.6 Hemato-biochemical parameters

The counting of the red blood cells (RBCs) and total white blood cells (WBCs) was conducted according to Stevens. (1997). The microhematocrit method of Hesser. (1960) was used to detect hematocrit (HCT). Hemoglobin was measured according to Lee et al. (1999). RBC indices were calculated according to the formula suggested therein. Blood in the non-heparinized tubes was allowed to clot at 4°C . Then, the blood was separated through centrifugation at 5,000 rpm at 4°C for 20 min to isolate the serum. Biochemical parameters [e.g., glucose, albumin, total protein, globulin, creatinine, aspartic aminotransferase (AST), alanine aminotransferase (ALT), urea, and uric acid] were estimated using kits (Biodiagnostic, Egypt).

2.7 Erythrocyte morphology and nuclear abnormalities

The blood smears were fixed in absolute methanol, air-dried, and stained with 6% Giemsa stain according to the method proposed by Hamed et al. (2019b). According to staining quality, slides were selected, coded, randomized, and scored by one person according to Al-Sabti and Metcalfe. (1995) and Schmid. (1975) using an Omax microscope with a 14 MP USB Digital Camera (CS-M837ZFLR-C140U, A35140U3; China).

2.8 Antioxidant biomarkers

Three fish from each group were randomly selected and anesthetized using ice to lessen stress (Wilson et al., 2009). The serum was separated to measure enzyme activity. Antioxidants kits from Biodiagnostic Company, Cairo, Egypt were used to estimate total antioxidant capacity (TAC), malondialdehyde (MDA), total peroxides (TPX), superoxide dismutase (SOD), and catalase (CAT) according to the procedure of (Nishikimi et al., 1972; Ohkawa et al., 1979; Aebi 1984; Koracevic et al., 2001; Waseem and Parvez 2013), respectively.

2.9 Statistical analysis

The homogeneity of variance was assumed for raw data. Moreover, in the absence of interactions, the pattern of variations was recorded by one-way ANOVA considering Tukey-HSD test for multiple comparisons using IBM-SPSS package version 21 (IBM-SPSS, 2012) at 0.05 significance.

TABLE 1 The chemical composition, trace elements, and REEs of the rock samples in this study.

Major elements (weight %)

Sample number	I	II	III	IV
SiO ₂	59.44	58.18	75.34	66.80
TiO ₂	1.033	1.167	0.112	0.547
Al ₂ O ₃	13.92	14.77	12.97	14.70
Fe ₂ O ₃	7.184	3.837	1.251	1.714
FeO	2.186	5.624	0.395	2.537
MnO	0.222	0.245	0.020	0.076
MgO	0.564	1.068	0.165	2.100
CaO	2.356	3.395	1.168	3.756
Na ₂ O	7.530	7.580	4.530	4.940
K ₂ O	3.910	2.681	3.276	1.912
P ₂ O ₅	0.223	0.335	0.034	0.118
Total	98.57	98.88	99.26	99.19
Trace elements (ppm)				
Sc	12.05	11.83	1.110	9.720
Ti	6652	6311	593.0	3110
V	19.21	2.310	6.110	87.16
Cr	11.18	9.280	231.0	146.0
Co	4.210	3.770	1.730	12.29
Ni	3.890	0.810	8.320	39.89
Cu	8.830	4.770	12.90	18.10
Zn	126.0	174.0	24.00	67.00
Ga	31.50	32.00	17.28	18.02
Ge	2.500	1.770	1.590	1.190
Rb	58.00	71.00	89.00	65.00
Sr	100.0	348.0	162.0	335.0
Y	43.00	72.00	24.00	19.00
Zr-RFA	288.0	746.0	228.0	167.0
Nb	65.00	6.690	5.130	11.30
Mo	4.080	6.310	1.880	1.550
Sn	3.490	0.150	0.160	0.150
Sb	0.150	0.590	0.730	1.310
Cs	0.670	987.8	657.8	476.9
Hf	6.860	11.77	3.900	1.770
Ta	3.660	1.120	3.450	0.870
W	1.810	0.060	0.260	0.270
Tl	0.190	6.110	9.850	7.420
Pb	7.200	0.020	0.030	0.040
Bi	0.110	14.93	13.25	3.890
U	5.840	3.110	3.720	1.480
Ba	1556	97.00	24.40	14.50
Rare Earth elements REE (ppm)				
La	47.0	193	47.8	29.2

(Continued in next column)

TABLE 1 (Continued) The chemical composition, trace elements, and REEs of the rock samples in this study.

Major elements (weight %)

Sample number	I	II	III	IV
Ce	89.0	22.0	5.57	4.04
Pr	11.8	83.3	21.3	16.8
Nd	49.7	17.2	4.37	3.70
Sm	10.4	5.37	0.95	1.04
Eu	4.38	16.5	3.70	3.64
Gd	9.37	16.7	4.07	4.07
Tb	1.47	2.39	0.65	0.57
Dy	7.76	2.60	0.84	0.67
Ho	1.48	7.29	2.46	1.89
Er	4.11	0.96	0.36	0.27
Tm	0.55	5.83	2.22	1.62
Yb	3.69	0.83	0.31	0.24
Lu	0.60	10.1	0.73	0.55

2.10 Ethical statement

The Research and Ethical Committee of the Faculty of Science at Assiut University approved the experimental design of this study.

3 Results

3.1 Physicochemical analysis

3.1.1 XRF

The contents of the major elements, trace elements, and REEs of the samples are tabulated in Table 1. It was observed that silica (SiO₂) and alumina (Al₂O₃) were the major components of all the samples, with percentages above 72.95%. Other components, such as TiO₂, Fe₂O₃, FeO, MnO, MgO, CaO, Na₂O, K₂O, and P₂O₅, were also variable. Still, the percentages of these components were lower than 8%. Sample III had the highest silica content, at about 75.34%, while sample II contained the highest alumina content, at approximately 14.77%. Meanwhile, the sample I had the highest hematite (Fe₂O₃), and sample VI had the highest CaO content (3.76%). A total of 31 trace elements were found, but seven of them, including Ti, Cr, Zn, Sr, Zr-RFA, Cs, and Ba, had significant contents over 100 ppm. The trace element with the highest content was Ti, with 6.65 ppm found in sample I. Sample II had the highest Cs content (987.8 ppm), while sample III had the highest Cr

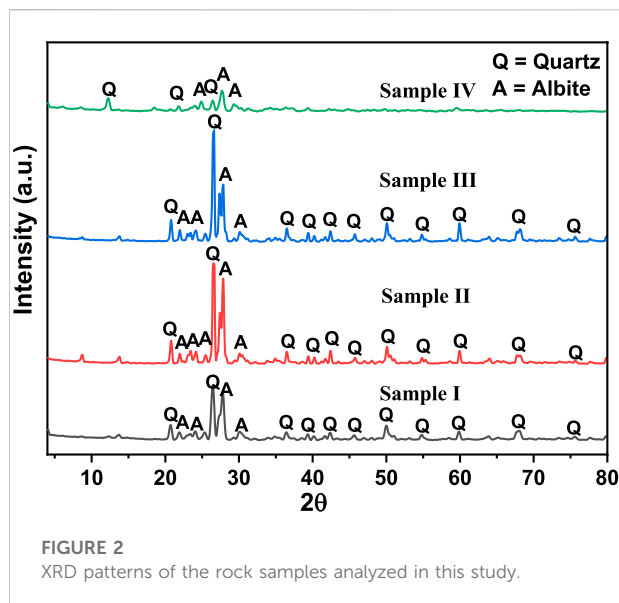


FIGURE 2
XRD patterns of the rock samples analyzed in this study.

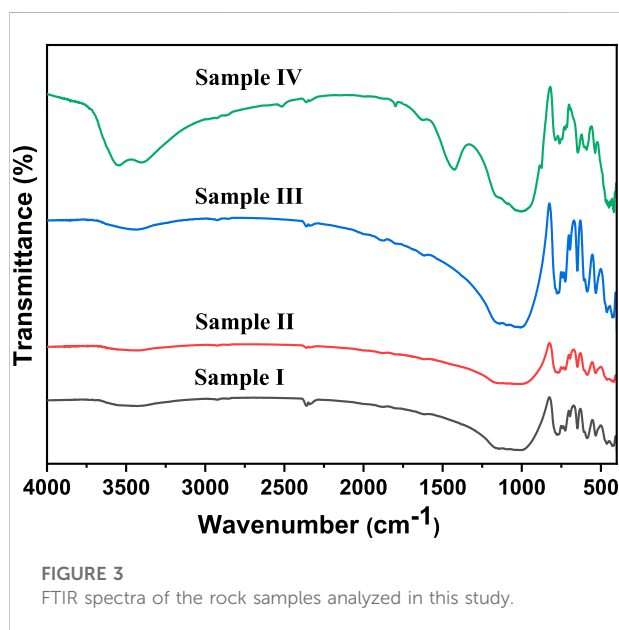


FIGURE 3
FTIR spectra of the rock samples analyzed in this study.

content (231 ppm). Furthermore, 14 REEs were also detected in the samples, but only three, including La, Pr, and Nd, had significant contents over 40 ppm. Sample II had the highest La (193 ppm) and Pr (83.3 ppm) contents, while sample I had the highest Nd content (49.7 ppm).

3.1.2 XRD

The XRD patterns of the samples are shown in Figure 2. All samples had reflections at 2θ values of 20.7° , 26.4° , 36.4° , 39.3° , 40.1° , 42.3° , 50.1° , 54.8° , 59.8° , 67.9° and 75.6° , indicating the presence of quartz (COD card no 00-901-1493) as the main component, which was in agreement with that observed from the

XRF data. At the same time, the peaks observed at 2θ of 13.7° , 21.9° , 23.4° , 24.1° , 25.4° , 27.6° , and 30.1° were characteristics of albite (COD card no. 00-900-9663). It was noted that sample III had the highest peak intensities, reflecting its high crystallinity. In contrast, sample IV had the lowest intensities of the XRD peaks, indicating that it had a low crystallinity. The average crystallite sizes were calculated using the Scherrer equation (Said et al., 2014) and were found to be 18.7 nm, 25.3 nm, 19.5 nm, and 15.8 nm for samples I, II, III, and IV, respectively.

3.1.3 FTIR

The FTIR spectra of the samples are shown in Figure 3. All had similar FTIR bands, except for sample IV, which had some slightly different bands. All samples had several bands at 3435 cm^{-1} , 1140 cm^{-1} , 1040 cm^{-1} , 1051 cm^{-1} , 1021 cm^{-1} , 777 cm^{-1} , 725 cm^{-1} , 694 cm^{-1} , 648 cm^{-1} , 586 cm^{-1} , 532 cm^{-1} , 463 cm^{-1} , and 425 cm^{-1} . The band located at 3435 cm^{-1} can be assigned to the stretching vibration of water molecules (Lyalina et al., 2019). The stretching vibrations of Si-O were detected at 1140 cm^{-1} and 1096 cm^{-1} , while the bands observed at 1036 cm^{-1} and 1015 cm^{-1} were the stretching vibrations of Al-O (Wang et al., 2009). The bands found at 777 cm^{-1} , 694 cm^{-1} , and 648 cm^{-1} could be attributed to the stretching vibration of Si-Si, and the bending vibration of O-Si-O, respectively (Xu and van Deventer, 2003). The asymmetric and symmetric stretching vibrations of M-O (M = Al, Si) were observed at 725 cm^{-1} and 425 cm^{-1} , respectively (Ma et al., 2015). The band located at 586 cm^{-1} could be due to the O-Si-(Al)-O bending vibration (Ramasamy et al., 2009). Meanwhile, the stretching and bending modes of the Fe-O bond in hematite were observed at 533 cm^{-1} and 463 cm^{-1} , respectively (Tadic et al., 2019). The FTIR spectrum of sample VI had two additional bands at 3546 cm^{-1} and 1428 cm^{-1} , corresponding to the stretching vibrations of OH groups (Lyalina et al., 2019) and the symmetric stretching vibration of unidentate carbonate (Imtiaz et al., 2013). The bands observed in samples III and IV were sharper than in samples I and II. This happened because samples III and IV had a higher SiO_2 content found in the XRF data (Table 1).

3.1.4 SEM

The SEM micrographs of samples II and IV are shown in Figure 4. The two samples had almost the same morphology. A mixture of blocky and flakey structures was observed, with an average size of about $1.2\text{ }\mu\text{m}$ and $0.6\text{ }\mu\text{m}$, respectively. Many fractures and pores were also observed.

3.2 Hematological parameters

The levels of all hematological parameters of *O. niloticus* with 15 days of alkaline rock exposure are recorded in Table 2. As mentioned in Table 2, the specimens exposed to alkaline rock had a pronounced decrease in their platelets. The count levels of WBCs, RBCs, blood hemoglobin concentration (Hb), and

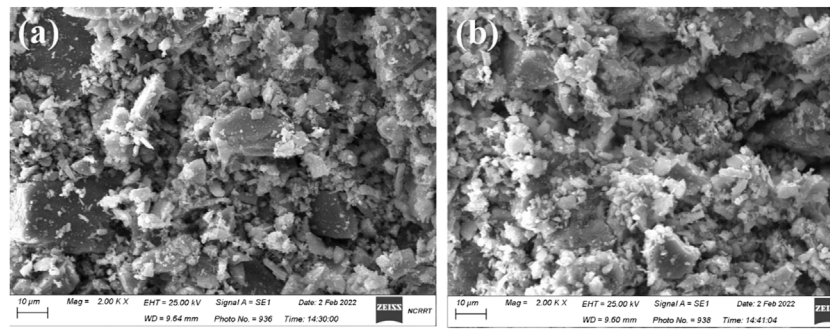


FIGURE 4
SEM images of samples (A) II and (B) IV.

TABLE 2 Effect of alkaline rock powder exposure for 15 days on the hematological parameters of juveniles of *Tilapia* (*Oreochromis niloticus*).

Treatment parameters	Control	100 μg/L sample I	100 μg/L sample II	100 μg/L sample III	100 μg/L sample IV
	Mean ± SE (min–max)	Mean ± SE (min–max)	Mean ± SE (min–max)	Mean ± SE (min–max)	Mean ± SE (min–max)
(RBCs) (Million/mm ³)	1.918 ± 0.0197 ^d (1.85–1.97)	1.628 ± 0.0054 ^c (1.61–1.64)	1.488 ± 0.025 ^{ab} (1.42–1.55)	1.5416 ± 0.0054 ^b (1.52–1.55)	1.445 ± 0.00957 ^a (1.42–1.48)
Hemoglobin (Hb) (g/dl)	8.45 ± 0.099 ^c (8.20–8.90)	7.933 ± 0.0557 ^b (7.8–8.10)	7.58 ± 0.040 ^a (7.5–7.7)	7.95 ± 0.0499 ^b (7.8–8.1)	7.8 ± 0.0258 ^b (7.7–7.9)
Ht (PCV) (%)	25.8 ± 0.3356 ^d (24.90–27.00)	24.35 ± 0.288 ^c (23.3–25.5)	23.17 ± 0.3124 ^b (22.2–24.14)	23.716 ± 0.270 ^{bc} (23.1–24.8)	22.023 ± 0.2754 ^a (21.14–22.7)
MCV (μm ³)	134.5 ± 1.3249 ^a (130.10–138.46)	157.93 ± 1.51 ^c (153.29–164.52)	155.94 ± 3.5413 ^c (145.45–170)	145.657 ± 1.7168 ^b (140.85–151.22)	152.396 ± 1.3289 ^{bc} (148.87–156.55)
MCH (Pg)	44.085 ± 0.859 ^a (42.35–48.11)	51.4657 ± 0.477 ^c (50.32–53.29)	51.023 ± 0.886 ^{bc} (48.7–54.23)	48.8259 ± 0.3553 ^b (47.56–50)	53.99 ± 0.44228 ^d (52.7–55.63)
MCHC (%)	32.78 ± 0.601 ^a (31.48–35.6)	32.603 ± 0.452 ^a (31.76–34.76)	32.758 ± 0.5354 ^a (31.07–34.68)	33.548 ± 0.516 ^a (31.45–35.06)	35.4483 ± 0.51243 ^b (34.36–36–92)
Platelets (Thousands/mm ³)	314.33 ± 0.614 ^c (312–316)	310.66 ± 0.421 ^{bc} (310–312)	301.33 ± 2.7527 ^a (289–306)	305 ± 3.376 ^{ab} (289–312)	298.666 ± 2.48551 ^a (289–304)
(WBCs) (Thousands/mm ³)	845.66 ± 2.36 ^b (841–857)	833.166 ± 3.07 ^b (824–845)	775.5 ± 21.5449 ^a (727–825)	839.5 ± 1.802 ^b (835–845)	794.66 ± 8.9243 ^a (775–821)
Lymphocytes (%)	88.166 ± 0.47726 ^b (86–89)	89.5 ± 0.2236 ^b (89–90)	89.5 ± 0.428 ^b (88–91)	86.833 ± 0.3073 ^a (86–88)	89.33 ± 0.333 ^b (88–90)
Monocytes (%)	2.66 ± 0.2108 ^a (2–3)	2.66 ± 0.2108 ^a (2–3)	2.166 ± 0.166 ^a (2–3)	3.5 ± 0.2236 ^b (3–4)	2.333 ± 0.21082 ^a (2–3)
Neutrophils (%)	7 ± 0.258 ^a (6–8)	6.66 ± 0.2108 ^a (6–7)	7.5 ± 0.3415 ^a (6–8)	7.5 ± 0.2236 ^a (7–8)	7.5 ± 0.22361 ^a (7–8)
Eosinophils (%)	2.166 ± 0.166 ^b (2–3)	1.166 ± 0.166 ^a (1–2)	0.833 ± 0.166 ^a (0–1)	2.166 ± 0.166 ^b (2–3)	0.8333 ± 0.16667 ^a (0–1)
Alterations (%)	1.04 ± 0.068 ^a (0–3)	2.63 ± 0.1238 ^b (0–6)	6.433 ± 0.157 ^d (1–10)	5.1266 ± 0.10288 ^c (2–8)	10.393 ± 0.135,077 ^e (7–15)

Data are represented as mean ± SE. Values with a different superscript letter in the same row for each parameter are significantly different ($p < 0.05$).

hematocrit (Ht) were significantly different from those in the control group ($p < 0.05$). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes, and monocytes had a significant increase in some treatment groups compared to the control group ($p < 0.05$). On the other hand, there were no significant variations in neutrophil cells in any of the groups (Table 2). Alterations in erythrocytes had a significant ($p < 0.05$) increase in rock-exposed groups.

3.3 Biochemical parameters

The levels of kidney function (creatinine), liver function (AST, ALT, and ALP), and other biochemical parameters (e.g., glucose, total protein, albumin, globulin, and A/G ratio) of *O. niloticus* specimens exposed to alkaline rocks for 15 days are shown in Table 3. In comparison to the control group, the levels of these parameters on the rocks-exposed fish had a significant increase ($p < 0.05$) in all treatment groups in a dose-dependent manner (Table 3).

TABLE 3 The effects of alkaline rock powder exposure for 15 days on the biochemical parameters of juveniles of *Tilapia (Oreochromis niloticus)*.

Treatment parameters	Control	100 µg/L sample I	100 µg/L sample II	100 µg/L sample III	100 µg/L sample IV
	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)
AST (µ/L)	54.95 ± 0.3879 ^a (53.8–55.9)	56.133 ± 0.2139 ^a (55.7–56.8)	63.066 ± 0.499 ^b (61.1–64.7)	54.46 ± 1.05 ^a (51.7–58.5)	65.55 ± 0.276 ^c (64.8–66.8)
ALT (µ/L)	28.733 ± 0.2155 ^b (28.2–29.5)	30.7 ± 0.34 ^c (29.5–31.5)	30.9 ± 0.25 ^{cd} (29.9–31.4)	27.6 ± 0.364 ^a (26.5–28.8)	31.83 ± 0.278 ^d (31.2–32.9)
ALP (µ/L)	24.933 ± 0.178 ^a (24.6–25.8)	28.35 ± 0.907 ^b (26.6–32.4)	28.366 ± 0.38 ^b (27.4–29.8)	26.4 ± 0.198 ^a (25.8–26.9)	28.5 ± 0.28 ^b (27.2–29.2)
Glucose (mg/dl)	103.1166 ± 1.0355 ^a (99.8–106.5)	109.28 ± 1.336 ^b (104.2–112.5)	116.35 ± 2.328 ^c (108.7–124.7)	110.743 ± 1.289 ^{bc} (105.36–113.2)	131.85 ± 1.8518 ^d (124.7–137.7)
Total protein (g/dl)	4.4 ± 0.068 ^a (4.2–4.6)	5.083 ± 0.087 ^b (4.8–5.4)	6.0166 ± 0.166 ^c (5.2–6.3)	5.016 ± 0.186 ^b (4.1–5.3)	6.383 ± 0.079 ^c (6.2–6.7)
Creatinine (mg/dl)	0.56 ± 0.016 ^a (0.54–0.64)	0.6216 ± 0.018 ^a (0.57–0.68)	0.7366 ± 0.01229 ^b (0.69–0.78)	0.568 ± 0.0237 ^a (0.52–0.66)	0.633 ± 0.02108 ^a (0.54–0.68)
Albumin (g/dl)	1.0916 ± 0.0279 ^a (1.02–1.2)	1.218 ± 0.022 ^b (1.11–1.26)	1.525 ± 0.0084 ^c (1.49–1.55)	1.193 ± 0.039 ^b (1.12–1.37)	1.486 ± 0.0217 ^c (1.42–1.55)
Globulin (g/dl)	2.125 ± 0.005 ^a (2.11–2.14)	2.206 ± 0.0229 ^b (2.14–2.28)	2.346 ± 0.020 ^c (2.32–2.45)	2.145 ± 0.012 ^a (2.10–2.19)	2.335 ± 0.0049 ^c (2.32–2.35)
A/G ratio	0.513 ± 0.0135 ^a (0.48–0.57)	0.552 ± 0.0086 ^a (0.52–0.57)	0.65 ± 0.0087 ^b (0.61–0.67)	0.556 ± 0.0187 ^a (0.52–0.64)	0.636 ± 0.008 ^b (0.61–0.66)

Data are represented as means ± SE. Values with a different superscript letter in the same row for each parameter are significantly different ($p < 0.05$).

TABLE 4 Effects of alkaline rock powder exposure for 15 days on the antioxidant parameters of the juveniles of *Tilapia (Oreochromis niloticus)*.

Treatment parameters	Control	100 µg/L sample I	100 µg/L sample II	100 µg/L sample III	100 µg/L sample IV
	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)	Mean ± SE (min-max)
TAC (µM/L)	1.061 ± 0.0047 ^a (1.052–1.08)	1.085 ± 0.0019 ^{ab} (1.08–1.09)	1.1105 ± 0.0078 ^b (1.09–1.14)	1.0735 ± 0.00209 ^a (1.07–1.08)	1.155 ± 0.0133 ^c (1.12–1.20)
MDA (nmol/ml)	4.1645 ± 0.0179 ^a (4.13–4.25)	4.2645 ± 0.0179 ^b (4.23–4.35)	4.44 ± 0.0064 ^c (4.42–4.46)	4.229 ± 0.005 ^b (4.21–4.25)	4.5296 ± 0.0277 ^d (4.45–4.63)
TPX (µM/L)	1.691 ± 0.0234 ^a (1.65–1.77)	1.77 ± 0.0042 ^a (1.76–1.78)	1.906 ± 0.0218 ^b (1.86–1.98)	1.758 ± 0.04028 ^a (1.65–1.88)	1.966 ± 0.0156 ^b (1.89–1.99)
OSI (%)	159.33 ± 2.007 ^a (155.08–167.30)	163.344 ± 0.5546 ^{ab} (161.76–165.43)	171.737 ± 2.3079 ^b (164.91–180.16)	163.81 ± 3.893 ^{ab} (153.06–176.19)	170.33 ± 1.537 ^b (165.97–174–96)
SOD (IU/L)	11.85 ± 0.0219 ^a (11.78–11.90)	12.3916 ± 0.105 ^{bc} (11.90–12.65)	12.56 ± 0.037 ^{cd} (12.42–12.65)	12.358 ± 0.03198 ^b (12.24–12.45)	12.67 ± 0.02113 ^d (12.62–12.75)
CAT (IU/L)	10.9 ± 0.0208 ^a (10.85–10.98)	11.316 ± 0.038 ^b (11.20–11.42)	11.48 ± 0.03134 ^c (11.42–11.63)	11.348 ± 0.0299 ^b (11.24–11.44)	11.59166 ± 0.0179 ^d (11.55–11.65)

Data are represented as means ± SE. Values with a different superscript letter in the same row for each parameter are significantly different ($p < 0.05$). TAC; total antioxidant capacity, MDA; monoaldehyde, TPX, total peroxides, OSI; oxidative stress index, SOD; superoxide dismutase, and CAT; catalase.

3.4 Antioxidant biomarkers

The activities of SOD, CAT, total peroxides (TPX), malondialdehyde (MDA), total antioxidant capacity (TAC), and oxidative stress index (OSI) of *O. niloticus* specimens exposed to alkaline rocks for 15 days are shown in Table 4. Compared to the control group, the activities of MDA, SOD, and CAT of the rocks-exposed fish exhibited a significant ($p < 0.05$) increase in all treatment groups in a dose-dependent manner. The activities of

TAC, TPX, and OSI had a significant increase ($p < 0.05$) only in a high concentration of two samples (100 µg/L) (Table 4).

3.5 Effects on erythron profiles

The erythrocytes of specimens in the control group had a well-shaped and normal size with their central nucleus, as shown in Figure 5A. However, the fish exposed to alkaline rock at a dose

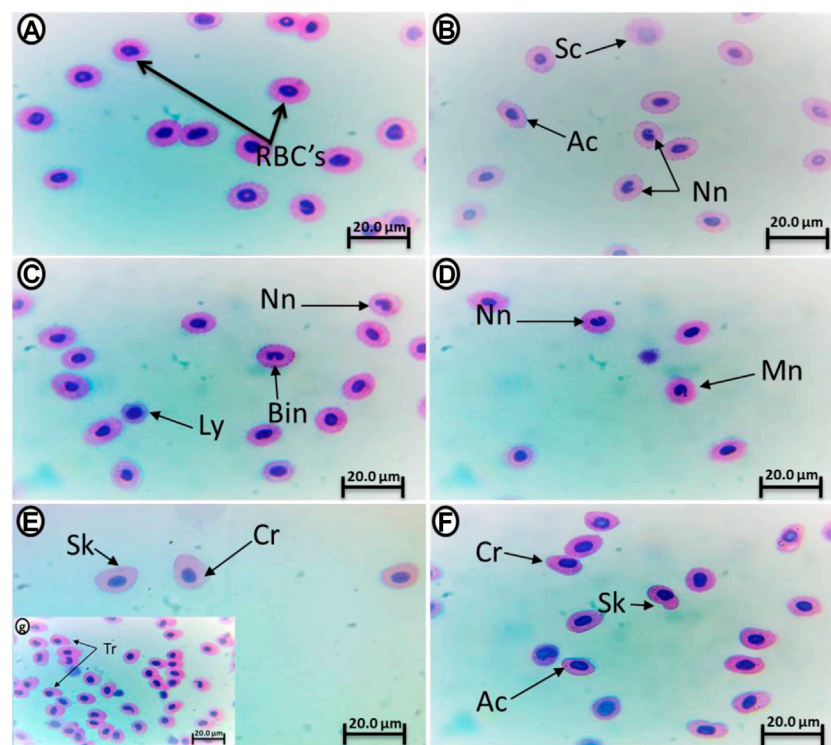


FIGURE 5

Represented blood smears from juvenile *Tilapia, Oreochromis niloticus*. (A) Control; (B–F) exposed to 100 µg/L alkaline rocks samples for 15 days; (RBCs) red blood cells; (Ac) acanthocytes (Cr) crenated cells; (Sk) Sickle cells; (Tr) teardrop-like cells; (Shc) Schistocyte; (Amc) Amoeboid cells, H and E stain, scale bar: 20 µm.

of 100 µg/L for 15 days had blood smears with poikilocytosis of the erythrocytes, as indicated in Figures 5B–F. This is confirmed by the presence of spinocyte, crenated, acanthocyte, kidney-shaped, and sickle cells. We also observed changes in RBCs containing tear-drop cells, lobed-nuclei cells, eccentric-nuclei cells, microcytes, and schistocytes.

4 Discussion

Rare earth elements can be reused and recycled through various technologies to minimize environmental impacts; however, there is insufficient data about their biological, bioaccumulation, and health effects (Romero-Freire et al., 2019). With rare earth elements' increasing use, environmental toxicity is becoming a concern, which may further harm human health. REEs' toxic effects are not fully investigated due to a lack of detailed reports (Malhotra et al., 2020). Trace elements and REEs usually sink into the aquatic environment as dissolved phases. They can be absorbed in sediments as well as aquatic plants and animals, resulting in multiple intake routes of inhalation, occupational exposure, and iatrogenic exposure. Research on the toxicity, mobility, and biomagnification of

REEs in water bodies can be conducted using aquatic biota, particularly fish. Among different indicators, bioindicators, such as the hematological and biochemical parameters of fish, can be used to justify aspects of water, including its quality (Saravanan et al., 2011). The decrease in the RBCs, Hb, HCT, and PL values of tilapia fish exposed to bulk alkaline rocks we observed can be attributed to the suppression of erythropoiesis (the progress of development by erythropoietin in the kidney), hemosynthesis, osmoregulatory dysfunction, or accession of the erythrocyte destruction rate of the hematopoietic organ, which may be caused by pollutants action (Oluah et al., 2020). Moreover, the decrease in PL values can be ascribed to the acceleration in RBC hemolysis and inhibition of erythropoiesis to the (Woryi et al., 2020). Furthermore, the significant reduction in RBCs resulting from the existence of many types of reactive oxygen species (ROS) (Dar and Borana 2014). These damaged RBCs may cause hypoxia, a reduction in HCT values, and Hb contents of the rock-exposed fishes (Abdel-Khalek et al., 2016). Such hypoxia suppresses aerobic glycolysis, which reduces the energy required for Hb production (Joshi et al., 2002). Even more, the detected lower values of HCT can be attributed to hemodilution and disrupted osmoregulation due to gill destruction (Kori-Siakpere et al., 2008).

The results of this study can be compared with other reported effects on fish under different stressors. For instance, the exposure of Nile tilapia to CuSO_4 and CuO nanoparticles can negatively influence hematological parameters (Soliman et al., 2021). Also, exposure to microplastics affects early juveniles blood parameters (Hamed et al., 2019b; Hamed et al., 2020). Alkaladi et al. (2015) and his research team reported a drop in the total count of WBCs of *O. niloticus* as a result of exposure to the of bulk alkaline, which may infect the lymphoid tissues of fish (Mukherjee and Sinha 1993). Furthermore, Nile tilapia and catfish exposure to nickel can reduce WBCs and affect the immune system (Ololade and Oginni 2010). Similarly, bulk alkaline rocks can decrease the WBC count from the accumulation of contaminants in the tissues.

Moreover, exposure to bulk alkaline rocks significantly influences other biochemical parameters, including liver functions, creatinine, glucose, total protein, albumin, globulin, and albumin to globulin (A/G) ratio depending on the dose rate. Higher enzyme levels are detected from the blood samples test, such as lactic dehydrogenase, AST, ALT, creatine kinase, and alkaline phosphatase, which correspond to damage to cell membranes (Banaee 2013). Such results are in agreement with other reported findings (Gill et al., 1992; Kazlauskienė and Stasiunaitė 1999; Monteiro et al., 2005; Heydarnejad et al., 2013; Pretto et al., 2014; Abdel-Khalek et al., 2015; Hamed et al., 2019a; Hamed et al., 2019b; Osman et al., 2019; Sayed et al., 2019; Soliman et al., 2019; Sayed et al., 2021a; Sayed et al., 2021b; Soliman et al., 2021; Hamed et al., 2022; Sayed et al., 2022). The variation in glucose levels is related to the glycogenolysis and gluconeogenesis processes occurring in the liver and kidney (Oner et al., 2008). Javed et al. (2017) showed that fish exposed to copper contamination might need to increase their protein levels to induce their immune system and tissue repair. In agreement with this work, the variation in the biochemical parameters has also been observed in goby (*Pomatoschistus microps*) after exposure to nickel contaminations (dos Santos Norberto 2014). However, the glomerular filtration rate and kidney dysfunction biomarker can be indicated by detecting creatinine and uric acid values (Banaee 2013; Abdel-Khalek et al., 2016).

Based on the results found by Hook et al. (2014), different aquatic environmental stressors are detectable using antioxidant biomarkers. In this study, there were significant increases in SOD, CAT, TPX, MDA, TAC, and OSI. Inflation in SOD and CAT after exposure to bulk alkaline rocks corresponds to the generation of ROS in fish. Such high SOD and CAT activities indicate that the liver is more likely to suffer from oxidative stress than other organs (Atli et al., 2006). This result is in coincidence with the reports published by Safari and his team (Safari 2016) that show that exposure to heavy metals can induce stress detection from the expression of genes encoding SOD and CAT to the detoxification of ROS (Rastgoo and Alemzadeh 2011). Similar results were detected in different types of fish, such as sturgeon, murrel, and tilapia (Atli and Canli 2007; Dabas et al., 2012; Safari 2016). OSI is

an indicator that can be used to investigate the interaction between different free radicals and the antioxidant system (Sayed et al., 2016a). Therefore, OSI has been investigated in different animal models, such as arsenite toxicity in goldfish (Bagnyukova et al., 2007) and methyltestosterone effects in *N. Tilapia* (*O. niloticus*) (Sayed et al., 2016a). Our result was suitable in compliance with the results found by Hamed et al. (2020) that the disclosure of microplastics may induce antioxidant damage in *N. Tilapia* (*O. niloticus*). The examined behaviors of antioxidant enzyme activity were in agreement with the results reported previously in studies involving different species exposed to heavy metal (Pedrajas et al., 1995; Paris-Palacios et al., 2000; Vieira et al., 2009; Wang et al., 2009; Tang et al., 2013; Hamed et al., 2019a; Sayed et al., 2019; Soliman et al., 2019; Sayed et al., 2021a; Sayed et al., 2021b; Soliman et al., 2021; Sayed et al., 2022). It has been reported in another study that the most significant finding was that the REE accumulated in zebra tissue after REE exposure was highly correlated to cytochrome c oxidase (CO1) and superoxide dismutase (SOD) were increased, while gene expressions of catalase (CAT) and glutathione-S-transferase (GST) were decreased (Hanana et al., 2017).

We investigated morphological erythrocyte amendment and nuclear distortions directed by the bulk alkaline rocks (Figure 5). As shown in Figure 5, there was an alternation in erythrocytes in the rock-exposed groups compared to the control. Comparable observations were recorded under the exposure of various aquatic species to other contaminants, such as copper sulfate and copper oxide nanoparticles (Soliman et al., 2021) and lead nitrate (Hamed et al., 2019b). Also, the rise in the morphological distortions of erythrocytes in catfish under disclosure to UVA was investigated (Osman et al., 2019). Even more, exposure to heavy can cause damage and mutation in DNA, reflecting in the quality of micronucleus and other alterations in erythrocytes, as previously studied (Osman et al., 2019; Soliman et al., 2019; Sayed et al., 2021a; Sayed et al., 2021b; Hamed et al., 2021; Soliman et al., 2021; Hamed et al., 2022). In another study conducted by Trifuoggi et al. (2017), a group of chlorides of 7 REEs were tested on two sea urchin species showed developmental defects and cytogenetic anomalies. Also, Kurvet et al. (2017) reported in his research that all studied soluble REEs were toxic to bacteria and protozoa. The mechanism of the cell damage can be explained as During the action, REE might compete with Ca^{2+} for binding sites in biological systems, inhibiting calcium channels in cell membranes and affecting the work of cells and tissues (Dave and Xiu 1991).

5 Conclusion

In conclusion, the bulk alkaline rocks induced changes in biochemical, antioxidant, and hematological parameters in *Nile tilapia*. Exposure to the bulk alkaline rock compounds also caused poikilocytosis and nuclear abnormalities of RBCs. This draws our attention to the seriousness of climatic changes, the

potential erosion of rocks, their access to water, and consequent effects on aquatic biota.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Assiut University.

Author contributions

Experimental design: ES and AS. Experiment and analysis: ES, MH, AE, MA, and AS. Data interpretation: ES, MH, AE MA,

and AS. Writing and revision: ES, MH, AE, MA, and AS. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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