Studies on behavioural, blood metabolites and biochemical composition of *Clarias gariepinus* exposed to quarry particles

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ABSTRACT

In fish, exposure to pollutants can induce increase or decrease in haematological and biochemical composition. Haematology, biochemical and behavioural parameters of *Clarias gariepinus* exposed to quarry dust were examined. Quarry particles were collected and taken to the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago - Iwoye for sieving with tight perforated sieve to allow free passage of only the dust, then weighed for the experimental exposure. Two hundred *C. gariepinus* juveniles were obtained from Ministry of Agriculture fish farm, Ikenne, Remo, Ogun State at three weeks and six days of age and then acclimatized for 14 days at the Animal House. The fish were fed with commercial fish feed at 5% of their body weight twice daily. During the acclimatization, 80% of water in each tank was replaced daily. Quarry dust of 65 g, 150 g and 250 g were introduced in each allocated labeled tank. Behaviour of the fish species were observed and then sacrificed for the haematology study through recommended procedures. The fish size range between 579 and 590 cm in total length and 670 and 690 grams in total weight, while the average water temperature, pH, dissolved oxygen, electric conductivity and total dissolved solids was 28.0±0.1 ºC, 7.8±0.025, 3.05±1.3 ppm, 683.75±50 µscm^{-1} and 432.33±64 ppm respectively. Haematological parameters showed pack cell volume (PCV) and neutrophils to be significantly ($P < 0.05$) higher and white blood count (WBC) lower in the fish species. Meanwhile, lymphocyte, eosinophil, basophil and monocyte showed no significant difference in all the exposure. *C. gariepinus* blood biochemistry showed lower triglycerides level and higher cholesterol and high density lipoproteins, while levels of blood bilirubin, albumin, protein, urea, creatinine and alanine transaminase were higher. Thereby, quarry dust has harmful effects on fisheries and the findings shall serve as a baseline data for study other fish species.

Keywords: African mud catfish, aquatic toxicity, blood chemistry, metabolites, quarry dust

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1. INTRODUCTION

Red blood cell count is quite a stable index which fish body tries to maintain within the limits of certain physiological standards using various mechanisms of compensation. Studies have shown that when the water quality is affected by toxicants, physiological changes will be evident in one or more haematological parameters (AAFS, 2020; ANSI/ASB, 2021; Yanuhar *et al*., 2021; Dias *et al.,* 2023). Blood cell responses are important indicators of changes in internal and/or external animal environment. The changes depend on fish species, age, spawners sexual maturity and diseases (Neveen *et al.,* 2010; Haider and Rauf, 2014; Javed *et al*., 2016; Ko *et al*., 2019; Casanovas *et al.,* 2021; Manna *et al.,* 2021; Kanu *et al.,* 2023).

In fish, haematological parameters are more related to the responses of their body system. Fish live in a very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Ahmed *et al.,* 2020; Kondera *et al*., 2021; Michail *et al*., 2022). In fish, exposure to pollutants alters levels of haematological parameters. Blood tissue truly reflects physical and chemical changes occurring in organisms. Therefore, detailed information can be obtained on general metabolism and physiological status of fish in different age groups and habitats.

Early diagnosis is possible when evaluating haematological data, particularly blood parameters (Oluah *et al*., 2020 Docan *et al.,* 2018 Witeska *et al.,* 2023. Also, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Docan *et al.,* 2018; Fazio, 2019; Rohani, 2023). Previous haematological studies on nutrition (Rehulka, 2000; Dawood *et al*., 2020; Duman and Sahan, 2023), infectious diseases (Rehulka, 2002; Javed *et al.,* 2016; Kondera *et al*., 2020) and pollutants (Fazio *et al.,* 2019; Bojarski *et al*., 2022; Dias *et al.,* 2023; Kanu *et al.,* 2023; Rohani, 2023) brought knowledge that erythrocytes are major and reliable stress indicators in the various sources (Richard *et al.,* 2003; Khan et al., 2016; Won Shin *et al*., 2016; Ahmed *et al.,* 2020).

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012; Docan *et al.,* 2018). Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al*., 2007; Khan *et al.,* 2016; Akhtar *et al.,* 2021). Haematological studies are of ecological and physiological interest in understanding the relationship of blood characteristics to the environment (Corredor-Santamaria *et al*., 2016). This could be so useful for animals that are genetically resistant to certain diseases and environmental conditions (Mmereole, 2008; Isaac *et al*., 2013; Fredianelli *et al*., 2019).

Haematological parameters are blood and its constituents forming organs (Bamishaiye *et al*., 2009) which are good indicators of the physiological status of animals ((Ahmed *et al.,* 2020; Witeska *et al.,* 2023). Blood act as a pathological reflector of exposed animals' status to toxicant and other conditions (Olafedehan *et al*., 2010). As reported by Isaac *et al*. (2013) animals with high-quality blood composition are likely to show good performance. Blood examination offers the opportunity to investigate the presence of various metabolites and other constituents in animal body and it plays a vital role in the physiological, nutrition and pathological status of an organism (Gomułka *et al*., 2014; Javed *et al.,* 2016; Ismail and Mahboub, 2016; Mahboub *et al*., 2021; Bojarski *et al*., 2022).

According to Olafedehan *et al*. (2010) examining blood constituents provide important information for the diagnosis and prognosis of diseases in animals. Blood constituents change in relation to the physiological conditions of the animals' health (Togun *et al*., 2007; Khan *et al.,* 2016; Akhtar *et al.,* 2021). These changes are of value in assessing animal responses to various physiological situations (Ahmed *et al.,* 2020; Witeska *et al.,* 2022; 2023). Changes in haematological parameters are often used to determine various body health status and stresses due to environmental, nutritional and/or pathological factors (Grant, 2015; Docan *et al.,* 2018; Fazio, 2019; Witeska *et al.,* 2022). This study therefore assessed the haematological profile and behavioural responses of *Clarias gariepinus* exposed to quarry particles in relation to aquatic toxicity.

2. MATERIALS AND METHODS

2.1 Experimental Design

Quarry particles were collected from a quarry site behind Olabisi Onabanjo University, Ago – Iwoye and taken to the Laboratory in the Department of Zoology and Environmental Biology, Olabisi

Onabanjo University, Ago-Iwoye for further processing. The particles were sieved with tight perforated sieve to allow free passage of the quarry dust and retain other quarry materials and then weighed (65 g, 150 g and 250 g) separately for the experimental exposure. The quarry particles were firstly mixed with 0.5 Litters of water to enable easy mixing with the water in the fish tanks (Oladunjoye *et al.,* 2023). After which the quarry-water mixture was added at one end of the tank different from the fish normal feeding spot. The exposure was done three times in a week and the tank water was changed each time.

Two hundred (200) African mud catfish, *Clarias gariepinus* were obtained from the Ministry of Agriculture Fish Farm, Ikenne, Ogun State in March 2020, at three weeks and six days of age and then transported to the animal house, Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago - Iwoye. The fish were fed with commercial pelleted fish feed at 5% of their body weight twice daily and kept at about 28ºC with 12h: 12h light-dark circle for three weeks to adapt to laboratory condition prior to the experiment (Ndimele *et al.,* 2015; Oladunjoye *et al.,* 2022).

During the acclimatization period about 80% of the water (dechlorinated tap water) in each tank was replaced daily. The fish size ranged between 579 – 590 cm in total length and 670 – 610 g in total weight during the period. Water temperature, pH, dissolved oxygen concentration (DO), electric conductivity (EC) and total dissolved solids were measured daily (Temp: 28.0±0.1ºC, pH: 7.8±0.025, DO: 3.05±1.3 ppm, EC: 683.75±50 µs/cm and TDS: 432.33±64 ppm).

The acclimatized fish were divided randomly into four; control and three exposed groups. Each group contained 40 juvenile fish in a plastic tank measuring 100 × 35 × 50 cm (L × W × H) with a total volume of 100 L. The fish were divided into the groups exposed to 150 g (T_1), 65 g (T_2), 250 g (T_3) of quarry particles and control group exposed to distilled water only without any quarry mixture. The fish species were exposed three times in a week for a period of six (6) weeks. Quarry mixture stocks were prepared by mixing each measured quantity of the quarry particles with 0.5 litres of water and then stirred. The experimental tanks were filled with clean water and the fish, then the prepared quarry particles were added three times in a week. 100% of the tank water was changed and the dosing was immediately restored after each exposure.

2. Behavioural and Haematological Examination

The fish behavioural changes were observed daily during the experimental period physically and their morphological changes were measured and recorded weekly. The fish species were taken out individually using a small hand net and placed belly upward on a table to prepare them for blood samples collection. Blood sample of about 4 millilitres was collected from the caudal peduncle with the aid of a plastic syringe, 1 ml of the blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant for haematological studies, while 3 ml was transferred into a tube containing lithium heparin anticoagulant to obtain plasma for biochemical analysis. 0.38 ml of diluting fluid (1% glacial acetic acid tinted with genital violet) was measured and dispensed into a small tube, while 20 µl of well mixed EDTA anti-coagulated venous blood was added and mixed as suggested (Oladunjoye *et al.,* 2021).

The plasma obtained by centrifugation from the lithium heparinised samples were stored at 20 ºC until all the haematological values were measured following standard methods. Packed cell volume (haematocrit method) and haemoglobin (Hb) concentration (cyanmethaemoglobin method) were analysed within two hours after collection in line with Owagboriaye *et al.* (2022). Red blood cells (RBC) and White blood cells (WBC) were counted by Neubauer's improved haematocytometer using Hyem's and Turk's solution as a diluting fluid respectively. Packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean cell

volume (MCV) were measured as described by (Ayala and Kerrigan, 2023).
The plasma was analysed for triglyceride, urea and creatinine, alkaline phosphate (ALP), cholesterol, aspartate transaminase, albumin, total bilirubin and total protein using multi-analyzer instrument as described by Oladunjoye *et al.* (2023b). The data obtained were statistically evaluated using the Randox kits for each parameter respectively (Saluk *et al.,* 2017; Efeoglu Ozseker *et al.,* 2023; Handley *et al.,* 2024).

3. RESULTS

3. Behavioural Responses

The behavioural responses of *Clarias gariepinus* exposed to quarry particles is shown in Table 1. The control fish were active and fed as required all through the experimental period. Similar behavioural response was observed in the *C. gariepinus* exposed to the varying concentrations of quarry particles during the first week. During the first week of quarry dust exposure, all the fishes in the exposed groups (T_1, T_2 and T_3) swam actively and fed well, but later gasped for air and regurgitated feeds taken.
During the second week of exposure, T_1 fishes fed moderately, while the T_2 and T_3 groups rushed their feed. During the third week, all the exposed fish groups became hyperactive and gasped for air. Similarly, all the exposed fish groups were restless and gasped for air during the fourth experimental week.

3.2 Physico-Chemical Parameters of the Water Samples

Results of physico-chemical evaluation of water samples exposed to varying concentrations of quarry dust before and after exposure are presented in Table 2. Results showed no significant difference (P > 0.05) in the levels of electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), nitrate and ammonia in the control and all the experimental water samples before exposure.
After exposure, EC was significantly (P < 0.05) lower in the control water sample than other groups. However, TDS, DO, COD, BOD, nitrate and ammonia levels were not significantly (P > 0.05) different between the control and those exposed to the varying concentrations of the quarry dust. Results also showed that EC and TDS were significantly (P < 0.05) reduced in all the water samples after exposure. On the other hand, there were no significant (P > 0.05) differences recorded in the water levels of DO, COD, BOD, nitrate and ammonia after the exposure.

3.3 Haematological Parameters

The haematological parameters of *Clarias gariepinus* exposed to quarry particles are shown in Table 3. The levels of pack cell volume (PCV) and neutrophils were significantly (P < 0.05) higher in the T_1 group. Levels of PCV and neutrophils recorded in the control group were not significantly (P > 0.05) different from the T_2 group. On the other hand, white blood count (WBC) was significantly lower in the T_2 and not significantly (P > 0.05) different between the T_1 and control groups. There were no significant (P > 0.05) differences in the lymphocyte, eosinophil, basophil and monocyte levels recorded in the T_1, T_2 and control groups.

3.4 Blood Biochemistry Composition

The blood biochemistry composition of *Clarias gariepinus* exposed to quarry dust is presented in Table 4. Triglycerides level was significantly (P < 0.05) higher in the control group than T_2 and T_1 respectively. Similarly, cholesterol and high density lipoproteins (HDL) were significantly (P < 0.05) higher in the T_1 group. On the other hand, there were no significant (P > 0.05) differences in the activities of alkaline phosphate recorded in the quarry dust exposed groups and the control group.
Activities of aspartate transaminase were significantly (P < 0.05) lower in the control than other exposed groups. Similarly, alanine transaminase was significantly (P

< 0.05) lowest in the control group and significantly ($P < 0.05$) highest in the T_1 group. Levels of blood urea and creatinine were significantly ($P < 0.05$) lowest in the control group and not significantly ($P > 0.05$) different between T_1 and T_2 groups. On the other hand, blood albumin and total protein levels were significantly ($P < 0.05$) higher in the control group. However, blood albumin and total protein levels were lowest in the T_1 group. Levels of total bilirubin are not significantly ($P > 0.05$) different between the control and exposed groups.

Table 1: Behavioural responses of *Clarias gariepinus* exposed to quarry particles

Group	T_1	T_2	T_3	Control
Week 1	Fish swam actively and fed well at first; Later, fish gasped for air and regurgitated the feed taken	Fish swam actively but fed well at first; Later, fish gasped for air and regurgitated the feed taken	Fish swam actively and fed well at first; Later, fish gasped for air and regurgitate the feed taken	Fish were active and ate as required
Week 2	Fish fed moderately	Fish rushed their feed	Fish rushed their feed	Fish were active and ate as required
Week 3	Fish were hyperactive and gasped for air	Fish were hyperactive and gasped for air	Fish were hyperactive and gasped for air	Fish were active and ate as required
Week 4	Fish were restless and gasped for air	Fish were restless and gasped for air	Fish were restless and gasped for air	Fish were active and ate as required

Table 2: Physico-chemical parameters of water samples exposed to varying concentrations of quarry dust

		EC	TDS	DO	COD	BOD	Nitrate	Ammonia
Before Exposure	T_1 (150 g)	1000.50±63.99[a]	682.50±50.46[a]	0.60±0.14[a]	622.35±54.94[a]	291.49±16.19[a]	178.20±26.97[a]	217.41±13.83[a]
	T_2 (65 g)	1129.50±63.99[a]	779.00±50.46[a]	0.70±0.14[a]	600.86±54.94[a]	288.20±16.19[a]	177.28±26.97[a]	202.09±13.83[a]
	T_3 (250 g)	1044.00±63.99[a]	721.00±50.46[a]	1.20±0.14[a]	555.27±54.94[a]	233.88±16.19[a]	149.02±26.97[a]	191.26±13.83[a]
	Control	1032.00±63.99[a]	711.00±50.46[a]	1.20±0.14[a]	533.52±54.94[a]	247.67±16.19[a]	152.57±26.97[a]	185.78±13.83[a]
After Exposure	T_1 (150 g)	335.00±63.99[a]	226.00±50.46[a]	0.80±0.14[a]	593.54±54.94[a]	271.85±16.19[a]	191.21±26.97[a]	202.45±13.83[a]
	T_2 (65 g)	331.00±63.99[a]	222.00±50.46[a]	1.00±0.14[a]	576.28±54.94[a]	262.38±16.19[a]	188.34±26.97[a]	192.16±13.83[a]
	T_3 (250 g)	344.00±63.99[a]	231.00±50.46[a]	0.70±0.14[a]	612.49±54.94[a]	287.15±16.19[a]	212.48±26.97[a]	225.89±13.83[a]
	Control	280.00±63.99[b]	222.00±50.46[a]	0.80±0.14[a]	603.16±54.94[a]	276.92±16.19[a]	207.15±26.97[a]	201.38±13.83[a]
	F – value	259.592	194.933	1.046	0.224	0.655	3.47	0.42
	p – value	0.01*	0.01*	0.34	0.65	0.44	0.10	0.54

[ab]Means (±Standard error of mean) in the same column for 'before exposure' and 'after exposure having similar superscript are not significantly different at $p < 0.05$; *Values significantly different after the exposure

EC;- Electrical Conductivity, **TDS;-** Total Dissolved Solids, **DO;-** Dissolved Oxygen, **COD;-** Chemical Oxygen Demand, **BOD;-** Biological Oxygen Demand

Table 3: Haematological parameters of *Clarias gariepinus* exposed to quarry particles

	T_1 (150 g)	T_2 (65 g)	Control
[c]PCV	48.67±6.36[a]	39.00±2.31[b]	36.33±0.88[b]
[d]WBC	117333.33±9333.33[a]	96000.00±8717.80[b]	122333.33±3844.19[a]
Neutrophil	5.67±1.45[a]	2.67±0.76[b]	2.00±0.58[b]
Lymphocyte	94.33±1.45[a]	97.33±1.76[a]	98.00±0.58[a]
Eosinophil	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Basophil	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Monocyte	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

[ab]Means ± Standard error in the same row having similar superscript are not significantly different at $p < 0.05$
[c]PCV:- Pack Cell Volume [d]WBC:- White Blood Count

Table 4: Blood biochemistry composition of *Clarias gariepinus* exposed to quarry particles

	T_1 (150 g)	T_2 (65 g)	Control
Triglycerides	81.00±17.35[c]	126.00±14.18[b]	138.33±25.31[a]
Cholesterol	144.33±15.32[c]	213.67±9.60[a]	173.00±7.81[b]
High Density Lipoproteins	86.33±5.84[c]	136.67±4.91[a]	119.00±2.31[b]
Alkaline Phosphate	1.29±0.22[a]	1.12±0.06[a]	1.12±0.12[a]
Aspartate Transaminase	25.45±1.60[a]	27.98±0.94[a]	10.65±0.71[b]
Alanine Transaminase	7.70±0.46[a]	4.47±0.41[b]	2.61±0.08[c]
Urea	4.78±0.98[a]	3.47±0.93[a]	2.00±0.43[b]
Creatinine	0.30±0.03[a]	0.34±0.06[a]	0.13±0.04[b]
Albumin	1.09±0.01[b]	1.38±0.01[a]	1.54±0.17[a]
Total Protein	3.29±0.02[c]	3.68±0.08[b]	4.01±0.18[a]
Total Bilirubin	0.20±0.03[a]	0.21±0.04[a]	0.22±0.02[a]

[abc]Means ± Standard error in the same row having similar superscript are not significantly different from each other

4. DISCUSSION

Physico-chemical parameters of water samples exposed to varying concentrations of quarry dust showed no significant difference in the levels of electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), nitrate and ammonia concentrations might be due to non-varying environmental conditions and pH levels. The result was in conformity with water parameters ranges reported by Bolawa and Gbenle (2012) and Awoyemi *et al.* (2014) on water quality for good fish culture.

The fish species exposed to quarry dust initially swam actively and fed well, but later gasped for air and regurgitated foods taken while becoming hyperactive and restless conform with Ogundiran *et al.* (2007), Dawood *et al.* (2020), Oladunjoye *et al.* (2024) findings. The stressful display and respiratory impairment may be due to the dust effect which was similar to Khan *et al.* (2016), Won Shin *et al*. (2016), Ahmed *et al*. (2020) and Oladunjoye *et al.* (2024) reports. Also, the observed increase in the state of inactivity with increasing concentrations and exposure period agreed with the report of Dias *et al.* (2023), Kanu *et al.* (2023) and Rohani (2023) findings. Ivon (2016) reported the hardiness of *Clarias gariepinus* to be due to the presence of aborescent air-breathing organ. The result agreed with the observation of Boran *et al.* (2012), Capkin and Altinok (2013) and Bertucci *et al.* (2014) but the presence of these anti-nutrients was not to a level to induce pathological changes in the fish.

Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sub-lethal effects in fish exposed to different toxic substances (Haider and Rauf, 2014; Corredor-Santamaria *et al*., 2016; Khan *et al.,* 2016; Fredianelli *et al*., 2019; Oluah *et al*., 2020). Significant increase in MCV, MCH and MCHC observed in the haematological studies of *Clarias gariepinus* was in agreement with the report of Shah (2006) on short-term exposure of Tench fish (*Tinca tinca*). These alterations might be attributed to direct or indirect structural damages to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis.

Blood biochemistry of *Clarias gariepinus* exposed to quarry dust showed higher triglycerides, cholesterol, high density lipoproteins (HDL), blood albumin and total protein levels. Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. Javed *et al.* (2016) reported haematological indices (RBC count, concentration of haemoglobin and haematocrit) as indicator of secondary responses of an organism to irritants and concluded that mechanism of lead toxicity occurs by ion regulatory disruption. The reduction in WBC count of the treatment groups that was observed agrees with the report that the release of epinephrine during stress causes a decrease of leucocyte count, which shows the weakening of the immune system (Oladunjoye *et al.,* 2021; Owagboriaye *et al.,* 2022).

Researchers have reported a decrease in haemocrit and haemoglobin with increase level of ingredients (Simeon, 2001; Akinwande *et al.,* 2004; Boran *et al.,* 2012; Capkin and Altinok, 2013; Bertucci *et al.,* 2014; Ndimele *et al.,* 2015) where no significant ($P>0.05$) difference observed in fish fed with control and test diets. Akinwade *et al*. (2004) opined that a measurable increase in white blood count of fish or any animal is a function of immunity and animals' resistance to some vulnerable illness or disease. This increase might indicate that *C. gariepinus* had high immunity or resistance to disease.

High value of white blood cells and erythrocyte counts observed were also recorded which indicated high oxygen absorption and transportation capacity of the fish cells (Ko *et al*., 2019; Yanuhar *et al*., 2021; Duman and Sahan, 2023). It is well known that a reduced quantity and quality of erythrocytes and a decreased haemoglobin level lead to a deteriorated oxygen supply (Dawood *et al*., 2020; Manna *et al.,* 2021; Michail *et al*., 2022; Dias *et al.,* 2023).

In addition to the transport of oxygen, erythrocytes have other functional tasks in the body, an insufficient quantity and quality of red cells would therefore consequently have several additional effects on metabolism beyond the simple oxygen supply for tissue metabolism (Akhtar *et al.,* 2021; Oladunjoye *et al.,* 2023b).

Fish are often used as sentinel organisms for eco-toxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of Mutagens (Boran *et al.,* 2012; Fredianelli *et al*., 2019; Witeska *et al.,* 2023). Therefore, the use of fish as biomarkers and indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems (Corredor-Santamaria *et al*., 2016; Won Shin *et al*., 2016; Kanu *et al.,* 2023; Rohani, 2023).

5. CONCLUSION

It is evident that contamination of water with quarry particles slightly affects the physico-chemical parameters of water. However, quarry particles causes behavioural, blood composition and haematological disruption in African mud catfish; *Clarias gariepinus*. Therefore, a slight decrease in the haematological parameters with no negative impact on the health status of the fish species may still be harmful for human consumption. Moreso, the study can serve as a baseline data for the toxicity of the quarry dust and its bioaccumulation effects along health risk factors. Meanwhile, quarry activities close to water bodies and where biota were cultured should be adequately monitored.

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