Progress Report

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Project title: Development of a community-based approach of protection of fish and crab's biodiversity from disastrous effects of mining activities in DR Congo

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I. Introduction

As mining activities expand in DR Congo, especially in South Kivu province/Mwenga territory, impacts on fish habitats and their biodiversity will become increasingly prominent. Additionally, since waterbodies polluted by these ores and heavy metals are connected to lakes, this may result in a global impact on aquatic biodiversity and human health. This project intends to develop community-based management strategies that will enable the protection of the freshwater fish and crabs' diversity from disastrous effects of mining activities in South Kivu province, DR Congo.
In terms of activities, the fish and crabs' sample collection has been done and we have identified species using identification keys. *Oreochromis niloticus,* one of the fish species mostly found in Mwenga territory has been characterized morphometrically. After the identification was done based on identification keys, molecular biology tools have been used to confirm the identity of species. In addition, interviews and focus group discussions with fishermen, farmers and local population have been conducted to evaluate their perception about the effect of mining activities on the fish and crabs' yield, diversity, and revenues from farming/fishing activity. Strategies to protect fish and crab diversity against mining threats have been suggested during these focus group discussions. In the following lines we describe what has been done and the results we have achieved so far. Few activities remain towards the end of the current project.

II. Executive summary

This project was commissioned to study the impact of mining activities on the fish and crab biodiversity in Mwenga territory, South Kivu province, DR Congo. This progress report outlines the methodology of the activities conducted so far and their associated key findings. So far, the fish and crab sample collection, the collection of water parameters, and the mapping of mining sites and waterbodies in which fishing, or fish farming is practiced have been conducted successfully. In addition, we have organized focus group discussions in order to determine fishers' perceptions towards the effect of mining on the fish yield and diversity. 90 fish and 60 crab samples have been collected, 45 waterbodies characterized, 23 mining sites and 45 waterbodies mapped. The identification of fish and crab samples based on identification keys as well as histological assessments have been carried out. DNA extraction, PCR amplification and sequencing of fish samples have been conducted in order to identify them genetically. Interviews and focus group discussions to assess fishermen, farmers and local population perception about the effect of mining activities on the fish and crabs' yield and biodiversity have been also carried out. During these focus group discussion management strategies have been suggested by stakeholders. Finally, the empowerment of stakeholders including fishers, farmers, mining operators, environmentalists, government representatives and local communities who will be applying the defined community-based strategies for the protection of fish and crab biodiversity against mining activity threats will be done.

III. Project objectives

- To inventory the freshwater fish and crab species available in Mwenga territory, South Kivu province, DR Congo.
- To assess the effect of mining activities on the biodiversity of crab and fish species available in Mwenga territory, South Kivu province, DR Congo.

- ❖ To determine the population perception about the effect of mining activities on the fish and crab's yield and biodiversity as well as on the revenue of farmers/fishers,
- ❖ To develop and vulgarize community-based strategies that will enable the protection of the fish and crabs' biodiversity.
- ❖ To empower stakeholders including fishers, farmers, mining operators, environmentalists, government representatives and local communities who will be applying the defined community-based strategies for the protection of fish and crab biodiversity against mining activity threats.

IV. Methodology of the so far conducted activities

The project was officially launched on 1st February 2023 for 12 months of implementation with the involvement and participation of all stakeholders. Despite the challenges encountered, so far, the project has achieved the following activities and associated results are already available.

4.1.Activity 1: Fish and crabs' sample collection

The fish and crabs' sample collection were conducted from 25th February to 10th March 2023 in different areas with intensive mining activities in Mwenga territory, South Kivu province, DR Congo. Concerned areas include Mwenga center, Kamituga, Kibe and Kitutu. These areas have also a high hydrographic potential (Lubyala River, Zalya River, Elila River, Ulindi River, etc.), most of which are affluents of the Congo River with fish farming as one of the main activities conducted by the local population. Below figures show how close are rivers and ponds to some mining sites in Mwenga territory.

Figure 1: Chinese mining site near Zalya river

Figure 2: Children looking for gold in water canalizations near ponds.

The sample collection in these rivers and ponds was scattered across the whole Mwenga territory from Mwenga center to Kitutu. Fish samples were collected from ponds using nets upstream, along and downstream every waterbody. In natural waterbodies, live fish and crab samples were purchased from fishermen. Organs (liver, kidney and muscle) and blood samples were collected from very few fish samples. They will be used to assess the effect of mining activities on the histology and hematology of fish in the area (Toxicity assessment). 5ml Syringes and needles were used for blood collection.

Figure 3: Fish blood collection. **Figure 4:** Fish organs preserved in 10% formol for histopathology analysis.

4.2. Activity 2: Collection of water parameters, mining sites and waterbodies geographic coordinates

Water parameters including temperature, pH and conductivity were collected for every waterbody using a multiparametric probe (YSI).

Geographic coordinates (Altitude, latitude and longitude) were collected for every waterbody and mining site in Mwenga territory using the GPS (Garmin GPSMAP 64S GPSMAP 64S).

Figure 5: Water parameter collection using a multiparametric probe.

4.3. Activity 3: Identification of fish and freshwater crabs' samples

This activity is ongoing. We are using identification keys to identify the collected fish and crab samples based on phenotypic features (Appearance and morphometric features) and this will be followed by the genetic characterization.

The morphometric characterization of collected fish samples has been already done. This was done first of all on *Oreochromis niloticus* which represents the mostly farmed fish species of Mwenga territory and also the more frequent in natural waterbodies (rivers). The morphometric measurements were conducted in the Molecular Biology laboratory of the Evangelical University in Africa. For every fish 22 parameters were collected including the Total length (Distance measured from the tip of the mouth/ snout to the tip of the caudal fin), Standard Length (Distance from the anterior edge of the upper lip to base of the caudal fin), Head Depth (Broadest distance measured perpendicular to the Standard length from the upper part of the head to its base), Body Height (Measured perpendicular to standard length, from the anterior base of the anal fin and the dorsal fin (broadest part of the body), Head Length (Measured in the horizontal plane as the shortest distance between the most posterior part of the opercula edge, and the projection from the edge of the upper lip, Pre-dorsal Distance (Distance between the start of the dorsal fin and anterior edge of the upper lip), Pre-anal Distance (Shortest distance from the start of the anal fin to the anterior edge of the upper lip), Pre-pectoral Distance (Distance measured horizontally from the base of the pectoral fin to the tip of the upper lip, Pre-ventral Distance (Distance measured horizontally from the base of the ventral fin to the tip of the upper lip, Pectoral Fin Length (Distance between base of pectoral fin and distal tip), Ventral Fin Length (Distance between the base of ventral fin and its distal tip), Dorsal Fin Base Length (Distance from the first dorsal spine to the last dorsal ray), Anal fin length (Distance measured from the first anal spine to the last anal ray), Inter-orbital Distance (Shortest distance between the bony edge of both orbits), Eye Diameter (Shortest distance between the skins around both eyes), Snout Length (Measured in the horizontal plane as the shortest distance between the anterior border of the eye and the anterior edge of the upper jaw, Caudal Peduncle Depth (Measured as the least depth of the caudal peduncle), Caudal Peduncle Length (Measured from the tip of the anal fin base to the start of the caudal fin, Greatest Dorsal Spine Length (Distance measured from the base to the tip of the longest dorsal spine), 3rd Anal Spine (Distance measured from the base to the tip of the 3rd anal spine), Longest Anal Ray (Distance measured from the base to the tip of the longest anal ray), Post orbital length (Distance measured from the eye orbit to the first spine of the dorsal fin).

After collecting the morphometric data collection, R software version 4.1.2 was used to determine the morphometric characteristics of the collected fish (*Oreochromis niloticus*) and potential differences between them. To eliminate the effects of size differences, the morphometric measurements were transformed into proportions of standard and head length. All data from the sampled fish (*Oreochromis niloticus*) were subjected to the multivariate analysis. Principal component analysis (PCA) and discriminant analysis function (DFA) were then used to analyze the data (Hammer *et al.,* 2001).

4.4. Activity 4: Mapping of the different mining sites and surrounding waterbodies in Mwenga territory.

Geographic coordinates that were used to map the different mining sites and surrounding waterbodies (rivers and ponds) in Mwenga territory, South Kivu province was collected using the GPS (Garmin GPSMAP 64S).

4.5. Activity 5: DNA extraction, PCR amplification and sequencing of fish sample in order to characterize them genetically.

Molecular biology tools have been used to confirm the identity of species (D'amato et al., 2007). This has involved the DNA extraction from fish blood and fin using the DNeasy Blood and tissue extraction kit (QIAGEN, USA) and following the manufacturer's protocol. Briefly, 200 µl of blood was placed in a 1.5 ml eppendorf tube; 2µl of proteinase K and 2µl of lysis buffer were added, vortexed and incubated at 56°C for 10 minutes. Then 2 µl of ethanol were mixed with the solution. for DNA precipitation (Shams et al., 2014) The precipitated solution was pipetted into the DNeasy Mini Spin column placed in a 2 ml collection tube, then centrifuged at 8,000 rpm for 1 min (Shams et al., 2014). then two successive washes were carried out with two wash buffers (AW1 and AW2). To facilitate DNA elution, 2 µl of elution buffer (EB) was added and the eluted DNA was stored at -20°C for future use. The amplification of the extracted DNA has been conducted by PCR and commercial sequencing of purified PCR products was done at Macrogen in Netherlands.

4.6. Activity 6: Population perceptions about the effect of mining activities on the fish and crab yield, diversity and revenue of fishermen and farmers

Face to face interviews have been organized using a structured questionnaire. 90 fishermen, 90 farmers and 90 individuals from the local community have been randomly selected. Selected fishermen and farmers should have at least 15 years of experience in fishing/farming activity (To be able to compare the current state to the one of 20 years ago when mining activities were not intense).

The questionnaire to be used consisted of three main sections: 1. Respondent's general demographic background; 2. Respondent's general knowledge of mining activities and biodiversity; 3. Respondents' perception of the impact of mining activities on fish and crab yield, biodiversity, and the farmer's/fishermen revenue and livelihood of their families.

4.7. Development and vulgarization of community-based management strategies

5 focus group discussions, each consisting of 12 randomly selected participants in average, have been organized. The 12 participants included 4 fishermen and farmers, 2 mining operators, 2 environmentalists, 2 government representatives and 2 representatives of the local community in order to define mechanisms that can contribute to the protection of fish and crabs' biodiversity from the disastrous effects of mining operations. Mechanisms that have been defined will be vulgarized in the community through a workshop and social marketing.

V. Achieved results

5.1. Specific result 1: A total of 90 fish and 60 crab samples were collected. Only 25 blood and organ samples were collected in the whole area (As this required killing the fish/crab we could not kill so many for biodiversity preservation purposes).

Figure 6: Collection of fish in ponds.

Figure 7: Diversity of collected fish species.

Figure 8: Diversity of collected fish species.

Figure 9: A fish found dead in mercury residues from a nearby mining extraction site.

Figure 10: Diversity of crabs collected.

5.2.Specific result 2: Multivariate analyses of the Nile tilapia morphometric data

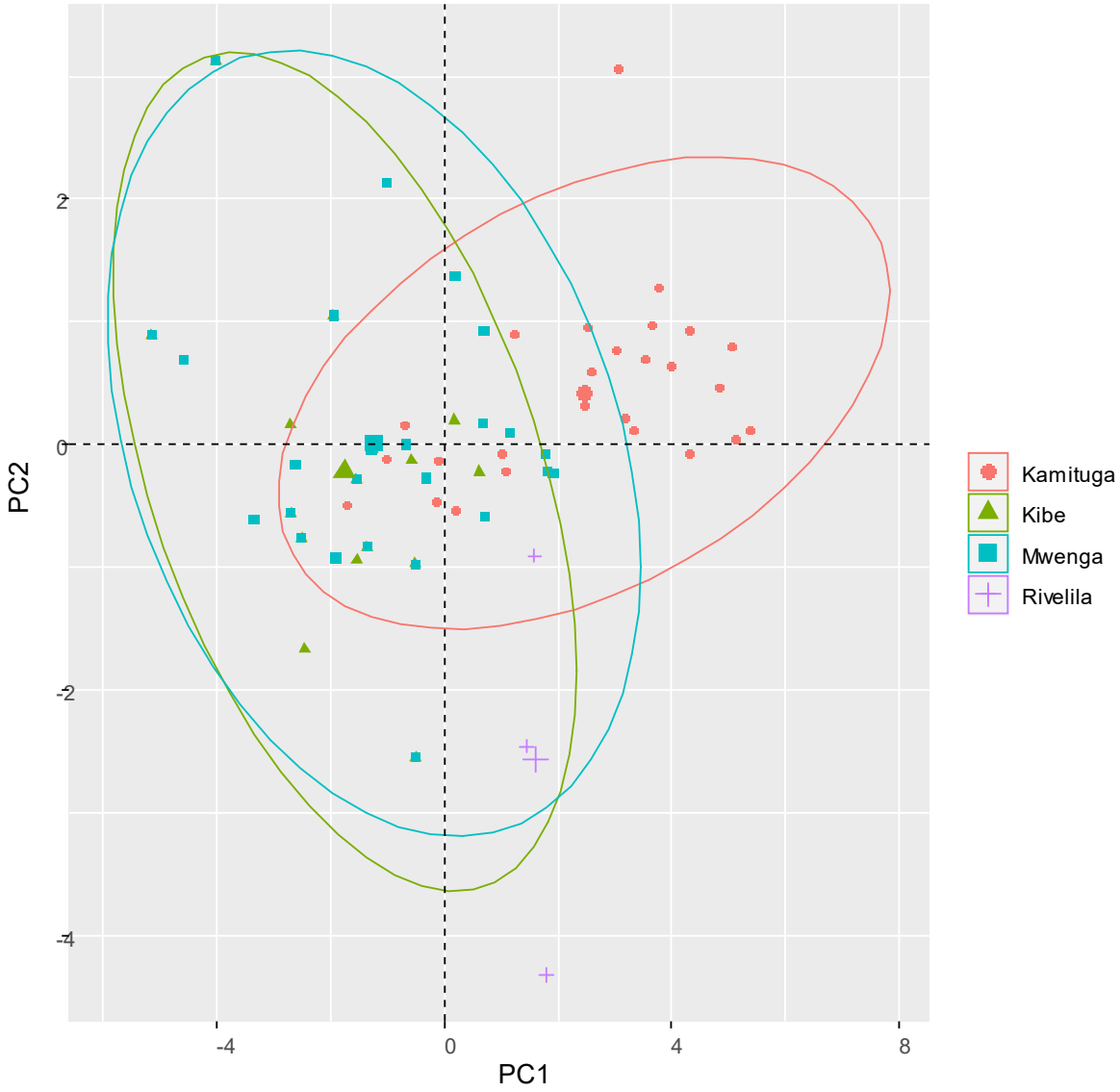

Figure 11: Principal Component Analysis of morphometric data of Nile tilapia sampled in Mwenga territory, South Kivu province, DR Congo.

The above figure shows separation between a group of Nile tilapia fish sampled at Kamituga and those from Mwenga, Kibe and Kitutu (Rivelila).

Individuals from these different sites partially intersect each other by overlapping.

5.3.Specific result 3: Map of the different mining sites and surrounding waterbodies in Mwenga territory.

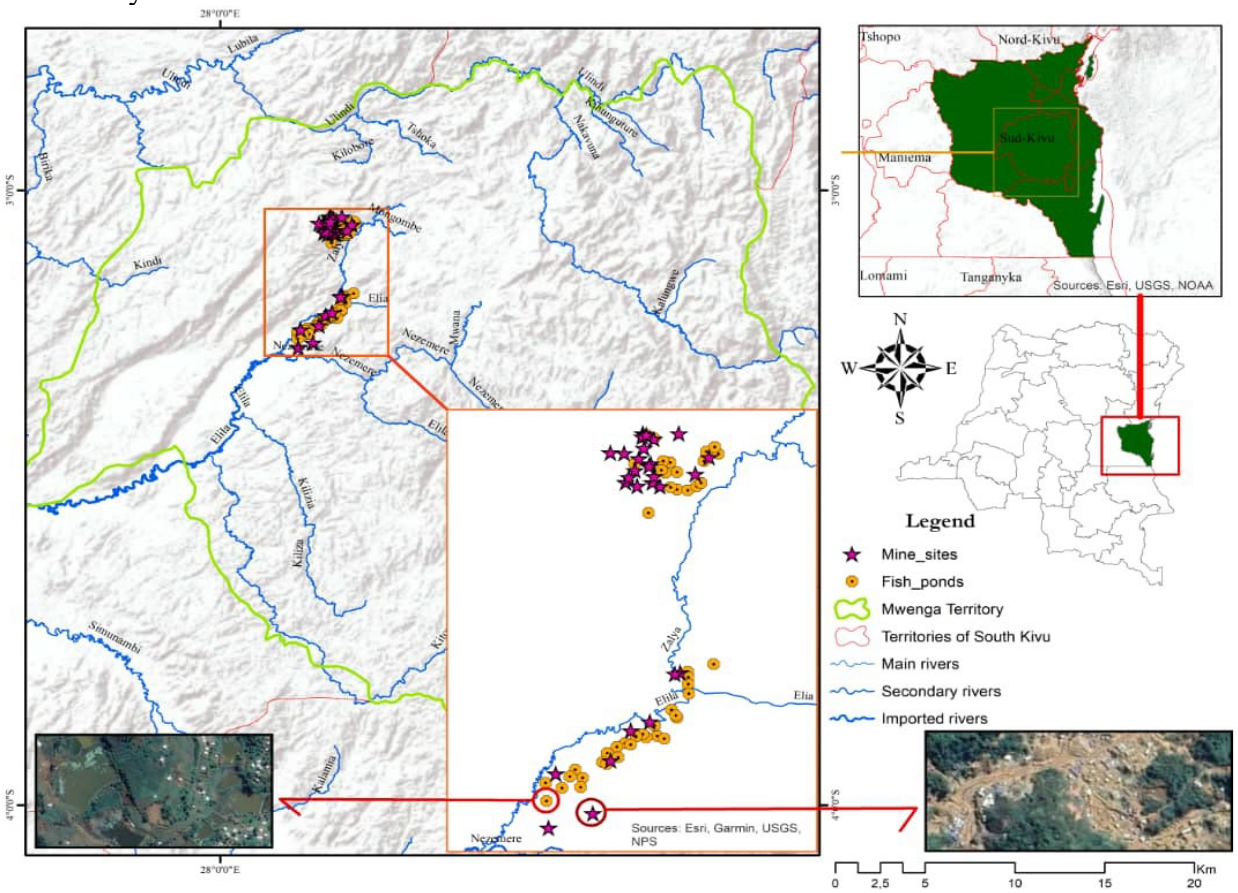

Figure 12: Map of mining sites and surrounding waterbodies in Mwenga territory, South Kivu, DR Congo

5.4.Water parameters

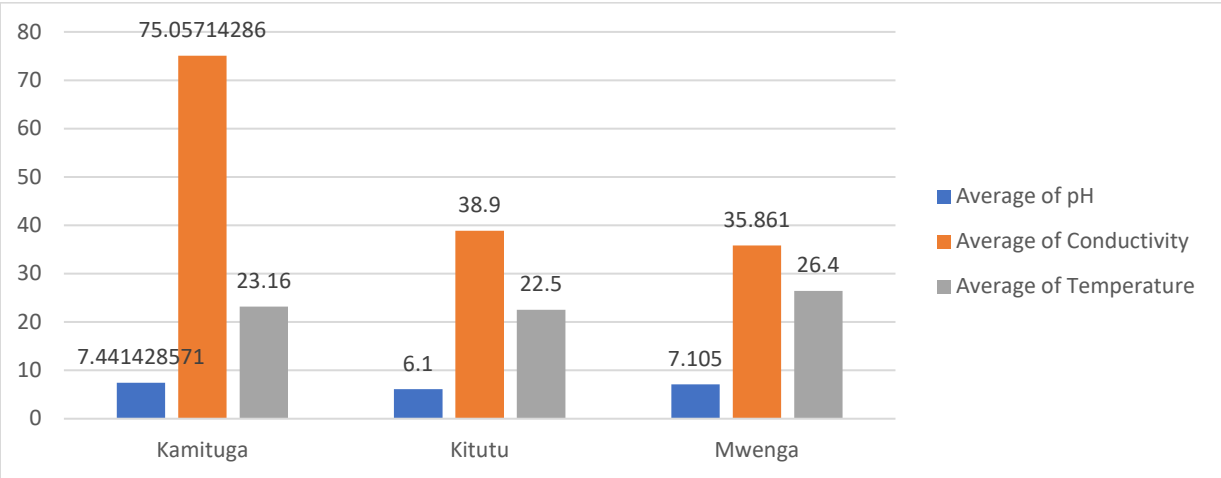

Figure 13: Average water parameters for waterbodies sampled in Mwenga territory, South Kivu, DR Congo

Figure 13 shows that waterbodies located in Kitutu have the lowest pH (acidic pH) and temperature. However, the lowest conductivity was observed in Mwenga territory. The lowest pH observed in Kitutu could be explained by the fact that there are so many mining sites in the area and most of them are interconnected with waterbodies in which fishing and/or fish farming are practiced.

5.5. DNA extraction and PCR

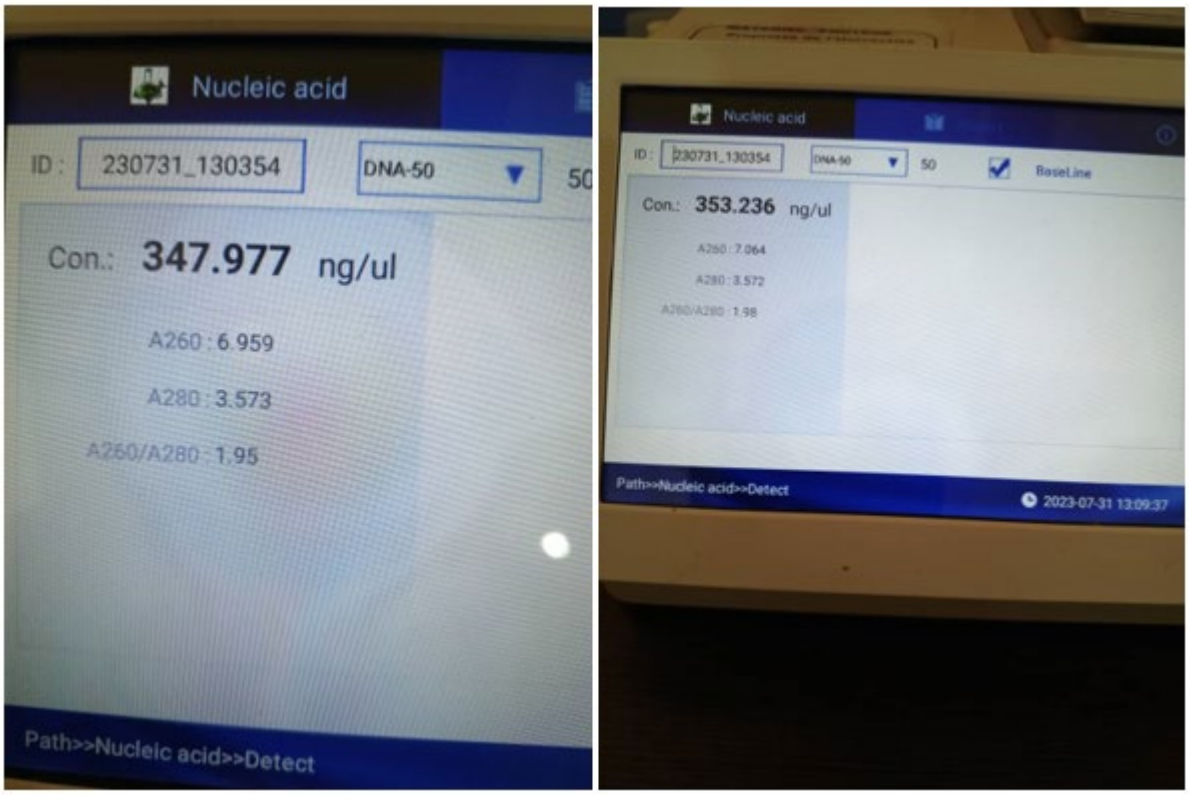

Figure 14: Sample DNA properties after extraction.

The DNA extracted showed high concentrations (above 200 ng/uL) and had a good ration of the absorbance at 260 and 280 nm (between 1.7 and 2.2).

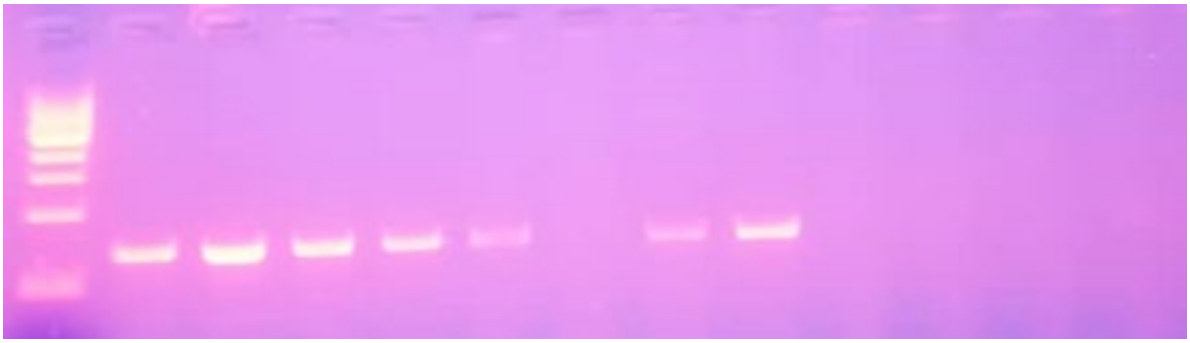

Figure 15: Gel image of PCR products after the amplification.

The amplification of the target gene has been conducted successfully. Following the amplification PCR products were sent via DHL to Netherland for sequencing (Macrogen). We have already received the sequencing data and currently we are conducting their analyses.

5.6. Histological analyses

Out of the 25 analyzed samples, 5 showed histological abnormalities. They are below described based on the sample id:

1. Kit2: Sections of striated skeletal muscle showing deposits of iron clods
2. Kib 5: sections of liver parenchyma with massive deposits of haemosiderin (iron)
3. KG16: sections of striated muscle without significant histological lesions and of liver parenchyma with discrete deposition of haemosiderin.
4. KG9: sections of striated muscle and fibrous tissue lined with respiratory-type epithelium with haemosiderin deposition.
5. KG15: sections of striated muscles, sections of liver parenchyma + massive deposition of haemosiderin (iron)

5.7. Population perceptions about the effect of mining activities on the fish and crab yield, diversity and revenue of fishermen and farmers

Eight species that were once present in the area have completely disappeared (Their names will appear in our publication whose write-up is ongoing). Under natural conditions, finding these species has become difficult, if not impossible. The species that remain show the same trend. If we compare the individuals obtained by fishermen in the last fifteen years with those of today, we find differences in values with higher values in years gone by than 15 years ago compared to today while fishing techniques remain the same. These principles are generally based on the installation of traps along the river. Similar perceptions have been made for crab species whose diversity keep on reducing in the study area. In terms of yields, a decrease has been mentioned by the local population for both fish and crab when comparing the current situation to the one of 15 years ago (Fish species current yield values and the ones of 15 years ago will appear in our publication whose write-up is ongoing).

5.8. Development of community-based management strategies

Figure 16: Focus group discussion.

Figure 17: Focus group discussion.

Community-based strategies to protect fish and crab diversity against mining threats have been suggested in the organized focus group discussions involving all the stakeholders.

Suggested strategies include:

1. The strict channeling of water used for fish farming (suggested by more than 80% of stakeholders). This water should not be in contact with any mining canalization.
2. Raise awareness: Conduct community education programs and workshops to create awareness of the detrimental impacts of mining on fish and crab populations and their habitats. By informing the community about the potential consequences, they can better understand the need to protect fish and their ecosystems.
3. Creation of local regulations: Collaborate with local authorities, fishery management agencies, and community members to establish regulations that specifically address mining activities and their impacts on fish populations. This can include restrictions on mining practices near water bodies or setting limits on pollutant discharge into rivers and streams.
4. Strengthening monitoring and enforcement: Empower local communities to monitor mining activities and report any suspicious or illegal mining practices that may harm fish and crab populations. By working closely with law enforcement agencies, community members can play an active role in ensuring compliance with environmental regulations.
5. Foster community-led conservation initiatives: Encourage the formation of community-based organizations or fishery cooperatives that aim to protect the local fish populations and their habitats. These groups can promote sustainable fishing practices, establish protected areas or fish sanctuaries, and actively engage in habitat restoration efforts.
6. Engage in dialogue and negotiation: Facilitate dialogue between mining companies, government agencies, and local communities to find common ground and develop mutually beneficial solutions. This can involve negotiating for responsible mining practices, mitigating negative impacts, and compensating communities for any damages caused to fish and crab populations.
7. Implement alternative livelihood options: Support the development of alternative income-generating activities for local communities, such as ecotourism or sustainable agriculture, which reduce the reliance on mining. By providing viable alternatives, communities may be more inclined to protect their fish populations and habitats.
8. Building capacity: Provide training and capacity-building programs to local communities, empowering them to monitor water quality, assess fish and crab populations, and implement sustainable fishing practices. Building local expertise will ensure the long-term protection and management of fish and their habitats.

VI. Key performant indicators

Table 1 presents activities so far conducted, associated specific results and indicators.

Table 1: Activities conducted, associated specific results and indicators.

Activity	Specific result	Initial status	Target	Final status
Fish and crabs' sample collection	90 fish and 60 crab samples collected	0	60 fish and 60 crab samples	90 fish and 60 crabs collected
Characterization of water parameters for the different waterbodies surrounded by mining sites	45 waterbodies characterized	0	Characterization of 15 waterbodies	45 waterbodies characterized
Mapping of mining sites and surrounding waterbodies containing fish/crabs	23 mining sites mapped and 45 waterbodies	0	15 waterbodies	23 mining sites mapped and 45 waterbodies
Identification of fish and crab species based on identification keys	60 fish and 60 crab samples identified	0	60 fish and 60 crab samples identified	60 fish and 60 crab samples identified
Histopathology analysis	25 samples analyzed	0	25 samples analyzed	25 samples analyzed
DNA extraction, PCR and sequencing	Extracted DNA showed high concentrations and good quality (ration of absorbance at 260 and 280 between 1.7 and 2.2). We also got good PCR products which have been already sequenced.	No genetic characteristic data available		Activity achieved successfully.
Assessment of the population perceptions about the effect of mining activities on the fish and	Eight species that were once present in the area have completely disappeared.	Population perception unknown		Activity achieved successfully.

crab yield, diversity and revenue of fishermen and farmers	In terms of yield, a decrease has been observed			
Development of community-based management strategies	8 community-based strategies have been suggested	No community-based strategies available		Activity achieved successfully
Drafting of the first manuscript				Completed. The manuscript will be submitted to the journal soon.
Empowerment of stakeholders				To be done
Vulgarization of the developed community-management strategies				To be done
Analysis of sequencing data				On going
Drafting of the second manuscript				To be done
Final report				To be done

VII. CONCLUSION

In conclusion, this project aimed to assess the effect of mining activities on the yield and biodiversity of fish and crab in Mwenga territory, South Kivu province, DR Congo. Most of the project activities have been conducted. These include the collection of fish and crab samples, mapping of mining sites and waterbodies in which fishing or fish farming are practiced, and the collection of water parameters for the different waterbodies used for fishing or fish farming, the identification of fish and crab samples based on identification keys and histopathological assessments, the mapping of mining sites and surrounding waterbodies containing fish/crabs, the DNA extraction, PCR and sequencing for the genetic characterization of species, survey to determine the fishers and farmers perception of the effect of mining activities on the fish and crab diversity, focus group discussions to develop community-based management strategies, the write-up of the first manuscript. Remaining activities include the empowerment of stakeholders, the vulgarization of the developed community-based strategies, the drafting the second manuscript and the final report.

APPENDICES

Appendix 1: Histopathological analyses.

Appendix 2: Zalya river water polluted by artisanal mining activities conducted nearby.

Appendix 3: Collection of water parameters in Lubyala river.