

Final Project Report

1. Contestant profile

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2. Project overview

Title:	Quarry biodiversity in focus – exploring the barcode of life
Contest:	Quarry Life Award 2016
Quarry name:	Quarry Nussloch (Germany)
Prize category: (select all appropriate)	<input checked="" type="checkbox"/> Education and Raising Awareness <input checked="" type="checkbox"/> Habitat and Species Research <input checked="" type="checkbox"/> Biodiversity Management <input checked="" type="checkbox"/> Student Project <input type="checkbox"/> Beyond Quarry Borders

Quarry Life Award 2016 project: Quarry biodiversity in focus – exploring the barcode of life

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1. Abstract

Our innovative project “*Quarry biodiversity in focus – exploring the barcode of life*” harnessed the power of DNA technology as analysis and science education tool to assess, with molecular resolution, the biodiversity in HeidelbergCement’s restored quarry in Nussloch, Germany.

To address the project’s research question, we established a unique collaboration between science educators of the European Learning Laboratory for the Life Sciences (ELLS) at the European Molecular Biology Laboratory (EMBL) and senior secondary school students from four different classes and their teacher at the Marie-Baum-Schule Heidelberg (MBS).

In order to assess the biodiversity of plants in different habitats within the restored quarry Nussloch, we employed a well-consolidated scientific technology – DNA barcoding. Using this technology, we analysed and compared plants based on their molecular DNA signature. More specifically, our project employed DNA barcoding to assess and compare plant biodiversity in two particular ecological niches in direct proximity: a heavy metal heap on a historical mining site and a rough pasture. The combination of field work with DNA barcoding and the use of modern biological databases providing open-access to DNA sequence data allowed us to increase our understanding of plant biodiversity in these specific ecological niches in the quarry. By successively carrying out all steps of the DNA barcoding process – from plant collection and molecular biology in the laboratory to bioinformatics analysis – the students’ interest in biodiversity-related STEM topics was boosted and raised their awareness of the importance of biodiversity management, particularly in restored mining sites.

Our aim has been to contribute knowledge and data to relevant biological questions relating to quarry biodiversity. The DNA sequence data we generated through our project work has been combined with geolocation data and allows tracking of the plant species identified with smartphone GPS accuracy. For the first time – and as an outcome of our student research project – a compilation of newly generated data sets from biological and geographical sources has been used to establish an online repository cataloguing plant biodiversity in the analysed quarry habitats. This open-access online resource could be extended in the future and might be used for HeidelbergCement’s targeted conservation and restoration approach.

The outcomes of this project are directly fostering the fascination for the uniqueness of quarry habitats. Based on the principle “*we can only protect what we know*”, our project has further raised public awareness of the importance of biodiversity conservation to safeguard the rich ecosystems in restored quarries.

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2. Final report

During the Quarry Life Award 2016 contest we have been harnessing the power of DNA barcoding as an analysis and science education tool to assess the biodiversity in HeidelbergCement's restored quarry in Nussloch, Germany. DNA barcoding is a well-consolidated scientific method that encompasses many concepts of modern biological research, applying DNA sequencing to identify organisms and infer their relatedness. Using this approach, we have involved young people in biodiversity-related STEM (Science, Technology, Engineering, and Mathematics) topics and raised their awareness of the importance of biodiversity management, particularly in restored quarries. Highlighting the principle “*we can only protect what we know*”, our DNA barcoding project stimulated independent student thinking across different levels of biological organization, linking molecular genetics to ecology and evolution. It integrated different methods of scientific investigation – from *in vivo* observations and *in vitro* biochemistry to *in silico* bioinformatics analysis – practising multidisciplinary research. Engaging the young students in scientific exploration in an industry-related setting highlighted the attractiveness of careers in science and business.

Our project “*Quarry biodiversity in focus – exploring the barcode of life*” employed the principle of DNA barcoding to assess the biodiversity and distribution of quarry plants growing on two different habitats in direct geographical proximity to each other. A rough pasture, i.e. nutrient-poor grassland, was compared to a heavy metal heap on a historical mining site with elevated levels of heavy metals (see Figure 1 and Figure 3 – Annex)

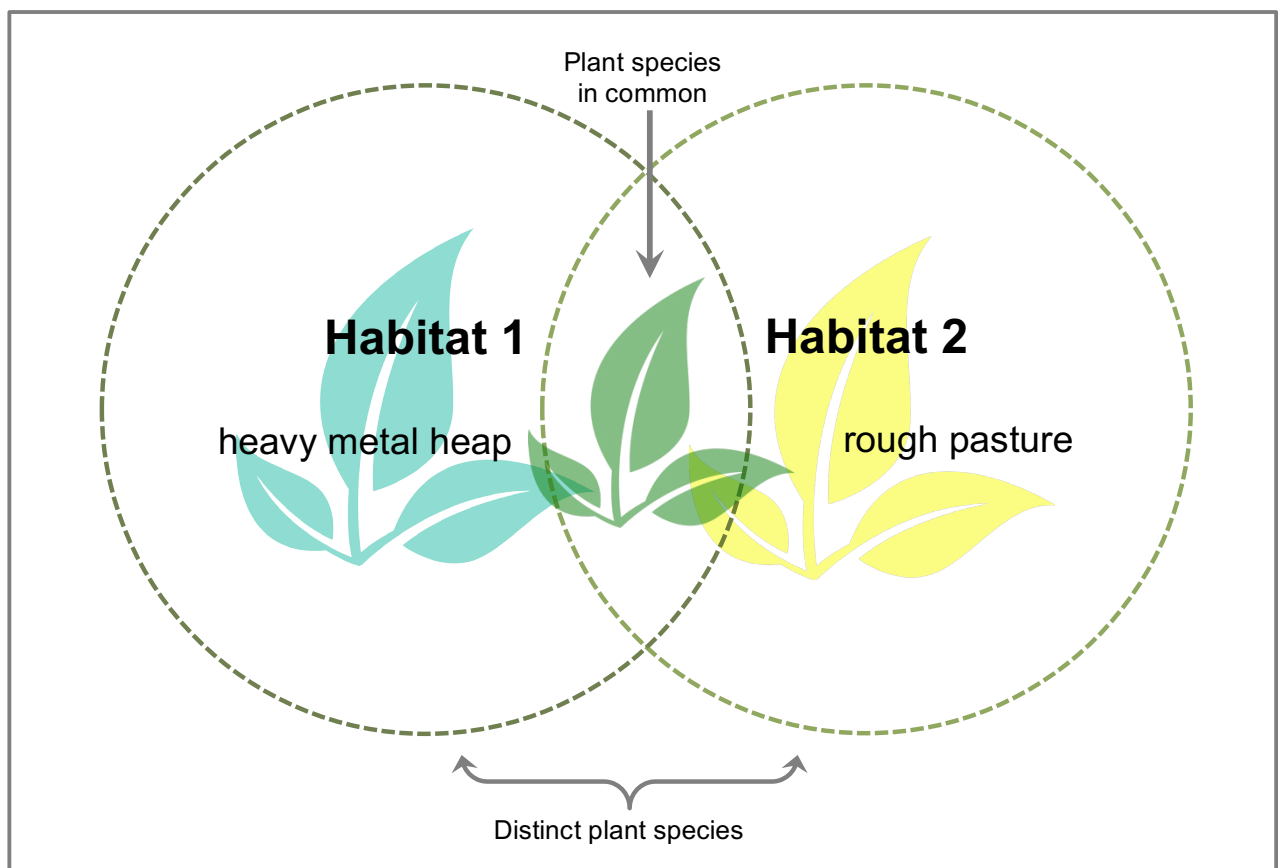


Figure 1 Comparison of two distinct plant habitats in the restored part of quarry Nussloch

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2.1. Rationale for our research question

The exploitation of natural resources, for example ore extraction in the hills marking the Rhine valley, has historically been carried out by the Celts and the Romans. The resulting depositions – and deposits of natural origin – can commonly be found as dumps containing increased concentrations of heavy metals (such as lead and zinc compounds). These habitats pose particular challenges to the vegetation and represent a very specific ecological niche that can be populated by plants that have developed strategies to cope with this rather non-favourable environment. Rough pastures, on the other hand, are found on soil with low nutrient levels such as those of thin alkaline soil made of limestone. They are an important habitat for a diverse range of plants, including grasses and herbs.

During the course of our project we have generated DNA barcode sequences from plants growing in both of these distinct habitats and identified them to species level by molecular and bioinformatics analysis. We aimed to compare plants which were phenotypically (observably) belonging to the same species but were growing on different substrates – with or without high levels of heavy metals. We were interested in investigating if during evolution distinct (sub)species might have evolved which are able to tolerate larger amounts of heavy metals in their substrates. Apart from gaining a better understanding of plant biodiversity in specific ecological niches of the quarry, we have been interested in identifying organisms that could potentially act as indicators for soil substrates, e.g. to identify the occurrence of increased quantities of heavy metals.

2.2. Involved groups and persons

The project has been set up as a unique collaboration between students, teachers, science educators and biodiversity experts. The group of senior secondary school students of the Marie-Baum-Schule Heidelberg (MBS) has been supervised by their biotechnology teacher Frank Luft. Field work in the restored quarry in Nussloch has been greatly supported and facilitated by Tina Gölzer, senior ecologist of HeidelbergCement. The school team collaborated with ELLS science educators and scientists to pursue experimental lab work and subsequent bioinformatics analysis in the vibrant scientific environment of the EMBL – Europe's flagship laboratory for the life sciences. Insights into their personal experience of the project are shared by participants in their personal statements (see Annex).

Prior to the start of the project, ELLS – in cooperation with HeidelbergCement – has been organizing an international teacher training course¹ on quarry biodiversity assessment by DNA barcoding. This forerunner course has helped to establish an experimental workflow tailored to quarry biodiversity and to acquaint the MBS teacher with the principles of DNA barcoding – a prerequisite to prepare his students for the project and to support them during all phases.

2.3. Methods

2.3.1. DNA barcoding and biodiversity

In order to investigate biodiversity in two distinct habitats (heavy metal heap, rough pasture) within the restored part of quarry Nussloch we decided to employ DNA barcoding. This technology can be used to differentiate organisms to species level by comparing specific stretches of DNA in their genomes. Species barcoding can take on a variety of perspectives, and the principle of DNA barcoding is routinely employed in a number of basic and applied scientific contexts, including tracking of the biological sources of commercial products, identification of product fraud (e.g. mislabeled food items), identification of novel compounds of medicinal value through bioprospecting or to extend our understanding of the responses of biodiversity to climate change. In the context of this project we applied DNA barcoding to take stock of the biodiversity in two specific quarry habitats and to compare the plant species distribution.

What exactly is DNA barcoding and how can it be used to address biodiversity challenges?

Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a short "DNA barcode" (about 600 nucleotides in length) is a unique pattern of DNA sequence from a particular gene or genomic region that can potentially discriminate between all living organisms. DNA barcodes allow non-experts

¹ ELLS LearningLAB course page: <http://wp.me/P3VwsP-5nw> and article about the course: <http://wp.me/p3VwsP-5r1>

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to objectively identify species – even from small organisms and damaged, or processed material. DNA barcoding therefore offers a refined way of bringing open-ended experimentation (inquiry) into formal and informal science education settings. In order to obtain good coverage and high identification accuracy of the plant samples, we followed the recommendation by the plant working group of the Consortium for the Barcode of Life² and analysed a combination of two barcodes (*rbcL* and *matK*) for each plant sample. Central to species identification by DNA barcoding is a biological database containing quality-controlled barcode sequences which can be queried against newly obtained quarry barcode sequences. The reference barcodes stored in such a database are regularly contributed by taxonomists who make sure that only accurate and referenced species data is entered into this ever-growing collection. We used the European Nucleotide Archive (ENA) for our analysis, which is a central repository of biological sequence data and is operated by EMBL's European Bioinformatics Institute (EMBL-EBI).

The team of the European Learning Laboratory for the Life Sciences (ELLS) at EMBL has been coordinating all steps and trained the students in core experimental techniques and in sequence analysis and identification tools in order to reach a satisfying and scientifically relevant research endpoint for their project. In addition, the team collaborated closely with a biodiversity expert of HeidelbergCement during their field trips to the quarry.

2.3.2. Experimental procedures

Figure 2 illustrates the three main steps in our DNA barcoding workflow: sample collection and processing, DNA barcode sequencing, and data analysis leading to species identification. Each step involves a specific set of processes to be performed (see Figure 2) and results in specific types of data that are generated, stored and used for subsequent analysis.

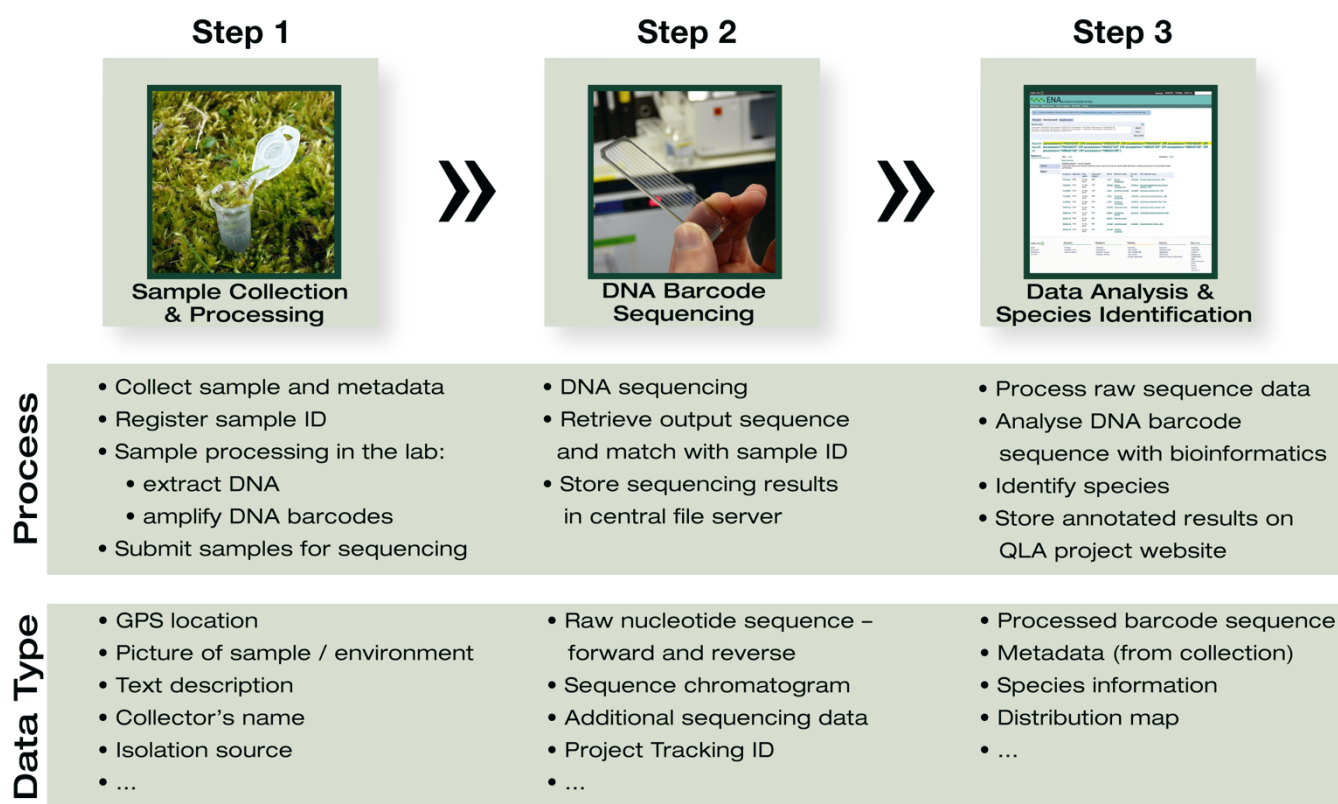


Figure 2 The project's DNA barcoding workflow, processes and data types

² CBOL Plant Working Group, Hollingsworth, P., et al. 2009. A DNA barcode for land plants. PNAS 2009 106 (31): 12794-12797. doi:10.1073/pnas.0905845106

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The following paragraph gives a short summary of the experimental steps involved. Detailed experimental protocols of each of the steps can be found on our project website at <http://emblog.embl.de/ells/qla2016>.

After obtaining appropriate plant material from the quarry, the plant's genomic DNA is isolated and the barcode region is amplified via an enzymatic reaction, the polymerase chain reaction (PCR). Subsequently, the presence of the barcode (i.e. the amplified PCR product) is confirmed via agarose gel electrophoresis, a method that separates the DNA fragments according to their length. After a clean-up step to remove unwanted reagents from the reaction, the PCR product is sent to a commercial sequencing provider. During the DNA sequencing process the exact succession of the DNA building blocks – the four nucleotide bases A (adenine), C (cytosine), G (guanine) and T (thymine) – is “read” and provided as a text file which can be used for further analysis. The unique barcode sequence of the plant sample can now be compared to known barcode sequences of plant species in a nucleotide database, the European Nucleotide Archive (ENA). All barcode data, species identifiers and associated information are finally submitted via the record submission form on ELLS' DNA barcoding portal and a complete entry for the respective plant is generated in the form of a web page.

Sample collection

For plant collection and documentation in the quarry we organized ourselves in teams of two and scanned the vegetation predominantly for plants that could be found in both habitats. Each group recorded information about their finds on a dedicated sample collection record sheet³. Information, such as a descriptive sample name, sample type and phenotypical characteristics (e.g. fresh leave, dried petal, shape, colour), sample image (sketch of the plant and/or digital picture), geographical coordinates, collection environment, date and collector's name, were recorded alongside a specific “sample identification number” (SampleID). This latter code was key to tracking the sample throughout the whole process. The SampleID consisted of a three-digit excursion code (e.g. 008), a single-letter sample-handler code identifying the collector (A=teacher, B=student or C=other) and a sample number (variable number of digits). A typical SampleID would therefore read like 008_B_61 and can be used to follow the plant sample up to its deposition in our [online repository](#).

DNA extraction

We have used a *Direct PCR* protocol⁴ to extract genomic DNA from plants. This involves cutting out discs from soft plant tissue, e.g. leaves, and placing the leaf discs in DNA extraction buffer. This buffer allows for cell lysis and DNA release from the cell's nucleus and the chloroplasts. The latter is particularly important as the barcode regions we analysed are located on the chloroplast DNA (cpDNA). After all extraction steps, the obtained mix of genomic and cpDNA can be used in the following PCR. Our detailed DNA extraction protocol with pictures illustrating all steps can be found on our project website⁵.

Polymerase chain reaction (PCR)

PCR allows to specifically amplify DNA sequences from a pool of genomic and chloroplast DNA. The specificity is obtained by employing short stretches of DNA (PCR primers) which are complementary to the start and end of the DNA sequences (barcodes) to be amplified. For the 50µl PCR reaction volumes we generally use approximately 100ng of DNA as PCR template. For each of the samples we perform two PCR reactions to amplify both *rbcl* and *matK* barcodes, respectively. The lyophilised PCR Master Mix contains dNTPs, DNA polymerase, buffers, stabilisers and BSA and is supplemented with an “additives mix” in solution (supplied with the kit), DNA template, primers and RNase-free distilled water.

We use the following thermal cycling conditions for PCR-based barcode amplification:

PCR protocol for *rbcl* barcode

Initial denaturation	94°C 3 min	1 cycle
Denaturation	94°C 30 sec	35 cycles
Annealing	56°C 30 sec	
Extension	72°C 45 sec	
Final extension	72°C 10 min	1 cycle
Preservation	4°C ∞	

³ Sample collection record sheet: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-1>

⁴ Jena Bioscience Direct PCR Lyophilised Master Mix

⁵ DNA extraction protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-2-1>

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PCR protocol for *matK* barcode

Initial denaturation	95°C 2.5 min	1 cycle
Denaturation	95°C 30 sec	10 cycles
Annealing	56°C 30 sec	
Extension	72°C 30 sec	
Denaturation	88°C 30 sec	25 cycles
Annealing	56°C 30 sec	
Extension	72°C 30 sec	
Final extension	72°C 10 min	1 cycle
Preservation	4°C ∞	

For a detailed PCR protocol, please visit the respective section on our project website⁶.

Visualisation of PCR products by agarose gel electrophoresis

In order to check which PCR reactions have been successful in amplifying target DNA, we use the FlashGel gel electrophoresis system. A fraction of each PCR reaction is mixed with loading dye which facilitates loading of the DNA-containing solution into wells of the agarose gel. High voltage is applied and due to the overall negative charge of its phosphate backbone the DNA migrates through the gel's matrix towards the plus pole. The DNA fragments, sorted according to their size, are then visualised by the activity of a DNA-intercalating substance which is contained in the buffer and which fluoresces upon exposure to light of 288nm wavelength. The samples to be processed further are selected according to their appearance and estimated size on the gel. The remaining volume of the corresponding PCR reaction is subjected to a purification step and submitted for DNA sequencing.

A detailed FlashGel electrophoresis protocol can be found on our project website⁷.

Bioinformatics analysis

The process of preparing a quality-checked input sequence for the search against database entries contained in the European Nucleotide Archive (ENA) is crucial for reliably identifying a plant sample to species level. The following paragraph summarizes the major steps involved. A more detailed bioinformatics analysis workflow is illustrated on the project website⁸.

After sending the generated PCR products to a commercial DNA sequencing service we obtain two sequences per DNA barcode (*rbcL* or *matK*) which span the barcode sequence in both directions. They are called the *forward* and the *reverse* sequences of each marker. Before the identity of the unknown plant species can be established by searching the barcode against the entries in ENA, the sequences of the forward and reverse reads have to be assembled into a single consensus sequence called a *contig*⁹. Assembling this contig sequence requires a) the conversion of the reverse sequence into its reverse complement, b) the alignment of the forward and reverse sequence reads, and c) quality checks and the assembly of the final consensus sequence. The sequence obtained can then be used to query the barcode reference sequence contained in ENA and to generate a list of closest hits for species identification. We have listed a detailed protocol on how to search the ENA database on our project website¹⁰ and explanations on the meaning of individual ENA result columns can be found under "Tools and Databases".¹¹

⁶ PCR protocol: <http://emblog.embl.de/ells/gla2016/what-is-dna-barcoding/step-2-2>

⁷ Agarose gel electrophoresis protocol: <http://emblog.embl.de/ells/gla2016/what-is-dna-barcoding/step-2-3>

⁸ Bioinformatics analysis protocol: <http://emblog.embl.de/ells/gla2016/what-is-dna-barcoding/step-3-1>

⁹ Definition of "contig" in our glossary: <http://emblog.embl.de/ells/blog/glossary/contig>

¹⁰ Database search protocol: <http://emblog.embl.de/ells/gla2016/what-is-dna-barcoding/step-3-2>

¹¹ ENA and other bioinformatics tools & databases: <http://emblog.embl.de/ells/gla2016/tools-and-databases/#ena>

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Online repository

We established a digital quarry species catalogue, putting the rich biodiversity within the restored quarry on display. This online resource can be freely accessed via our project website at <http://emblog.embl.de/ells/qla2016> and contains complete records of the species analysed and an interactive map showing where the plants have been collected. In addition, the portal also contains extensive information on the principle of DNA barcoding, detailed experimental protocols, activity reports and picture galleries of the project team's outdoor excursions, lab and computer practicals.

2.4. Results and discussion

During the course of our project, we have collected a variety of plant samples in the quarry Nussloch and processed them in the laboratory at EMBL. All of the plant samples have initially been subjected to amplification of *rbcL* and *matK* barcodes as we aimed to obtain datasets with most similar characteristics. For a fraction of the samples we observed difficulties in generating DNA barcode sequences due to one or more of the following reasons:

- DNA extraction from respective plant tissue was not possible/inefficient or resulted in degraded DNA
- Amplification via PCR was not efficient enough to obtain decent quantities of barcode-specific DNA
- DNA sequencing failed because of long poly-A/poly-T stretches within barcode region

In cases where a combination of both DNA barcodes could not be generated, we set out to obtain reliable DNA sequences for either of them and used those to proceed with detailed analysis.

Table 1 (Annex) shows the plant species identified for both habitats. It summarises record data that is featured in full detail on the respective plant sample record pages on our project website.¹²

Many of the plants we included in our analysis could be found in both habitats – the heavy metal heap and the rough pasture. As a result of the DNA barcodes used and the data currently available in the ENA biological database, it was possible to identify most of the plants to species level or even subspecies level.

We obtained good coverage and were able to find many of the plant species growing in both habitats. A fraction showed a distribution pattern exclusive for one single habitat. For example, within the quarry, the Eyebright (*Euphrasia* sp.) could only be found on the heavy metal heap. Extensive on site searches during excursions did not yield any *Euphrasia* in other parts of the quarry, including the rough pasture. This points to a possible preference of this particular plant species for growing on the heavy metal heap and it would be interesting to investigate further if this species could be used as an indicator plant for heavy metal containing substrates. However, as the overall time for our research was limited we could not follow up on this observation over a full vegetation period. This question could therefore be the subject of a future investigation. We also want to point out that we collected plants only on certain dates and therefore cannot rule out completely that *Euphrasia* species could still be found elsewhere in the quarry at other times of the year. In principle, such seasonal variations could play a role in vegetation patterns across the whole quarry, especially as habitats within the quarry often exhibit very unique characteristics. During the project excursions to the quarry we also observed that the “kinetics” in terms of development of vegetation in the two selected habitats varied significantly. This

¹² Full details of plant samples identified: <http://emblog.embl.de/ells/qla2016/plant-samples>

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effect has been further aggravated by the fact that 2016 has been a rather “wet” year. Especially in the first months of the year we observed long lasting periods of heavy rain and lower temperatures which probably also resulted in retarded vegetation. We assume that in spring time of a “typical” year we probably would have seen different growth and distribution patterns of plant species. Therefore, comparison of two or more vegetation periods could potentially help to underpin and further consolidate our current research findings.

It is well known that plants with the ability to grow on metalliferous soils – so called metallophytes – can accumulate metals in much greater concentration than plants that do not exhibit specific metal tolerance or resistance¹³. Since ancient times, certain metallophytes have therefore been used as indicator plants for ore prospecting. Some of the plant species we identified on the heavy metal heap are clearly belonging to families (e.g. Caryophyllaceae or Brassicaceae) that are known to contain species with heavy-metal tolerance or even resistance, such as *Silene*¹⁴ or *Dianthus*¹⁵. However, it needs to be noted that high metal tolerance is evolutionary not completely confined to specific taxonomic groups but can show differences even among populations within species – and may lead to separate ecotypes¹⁶. This would explain why the plant species distribution pattern in both areas was not completely exclusive for one or the other habitat. Taking into account this observation it would have also been interesting to combine our species analysis on DNA level with a comparative measurement of heavy metal concentration in plant tissue. Such an analysis could eventually support and expand our findings in the future. However, what we phenotypically observed with plants growing on the heavy metal heap have been pale-green to yellow leaves, sometimes in combination with dark spots on the leaves – pointing to heavy metal induced chlorosis and necrosis. Chlorosis is a condition where plants exhibit a disturbed chlorophyll production due to external factors such as excess of heavy metals¹⁷.

It is interesting to see that the plant communities we observed on the heavy metal heap can be categorized into typical stages¹⁸. Comparing previous observations¹⁸ with our catalogued list leads us to speculate that the heavy metal heap is in a transitioning stage between the “*Silene*” and the “*Euphrasia*” stages. This points to a steady reduction of heavy metals in the substrate and to an increase in fine soil which makes the area habitable for an even larger variety of rare plant species.

2.5. Added values of this project

2.5.1. Added value for biodiversity

During our project we have established a technical workflow to characterise large plant communities. This approach allows us to locate and identify rare plant communities which are unique to quarry habitats. In addition to phenotypic analysis, DNA barcoding can provide additional insights into the genetic basis of biodiversity. For example, this technology can help us to determine whether two plant samples belong to distinct subspecies or are rather to be considered as separate ecotypes of the same species. Supported by scientific data, we have generated a detailed picture of the composition of plants in the different quarry habitats, ensuring targeted and sustained conservation of biodiversity.

2.5.2. Added value for society

Our project has directly fostered the understanding and public awareness for the need of quarrying activities and the importance of subsequent restoration measures. The project participants experienced first hand that – besides the need for invasive quarrying activities – restored and renatured quarries provide a precious ecosystem for a diverse range of organisms. The group will act as ambassadors for the rich nature in quarries and its conservation. In addition, our communication strategy via social media, the official Quarry Life Award

¹³ Punz, W. (1995). Metallophytes in the Eastern Alps. With Special Emphasis on Higher Plants Growing on Calamine and Copper Localities. *Phyton* 35: 295-309

¹⁴ Colzi, I., Arnetoli, M., Gallo, A., Doumet, S., Del Bubba, M., Pignattelli, S., et al. (2012). Copper tolerance strategies involving the root cell wall pectins in *Silene paradoxa*. *Environ. Exp. Bot.* 78, 91–98. doi: 10.1016/j.envexpbot.2011.12.028

¹⁵ Punz, W. & Mucina, L. (1997). Vegetation on anthropogenic metalliferous soils in the Eastern Alps. *Folia Geobot.* 32: 283. doi:10.1007/BF02804008

¹⁶ Ernst, W. H. O. 2006. Evolution of metal tolerance in higher plants. *For. Snow Landsc. Res.* 80, 251–274

¹⁷ Prasad, M. N. V. 2004. Heavy Metal Stress in Plants: From Biomolecules to Ecosystems. Springer Berlin Heidelberg ISBN: 978-3-642-07268-0 DOI: 10.1007/978-3-662-07743-6

¹⁸ for a description of stages see: Schwermetallbelastungen durch den historischen Bergbau im Raum Wiesloch. Fachdokumente der Landesanstalt für Umweltschutz Baden-Württemberg: <http://bit.ly/2clqpTg>

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website and the web portal generated as part of the project allowed us to reach out to the general public and to specific audiences such as European school teachers and their students. We believe that the followers of our project have developed an appreciation for the rich biological diversity in quarries – a prerequisite to safeguard biodiversity for future generations.

2.5.3. Added value for the company

One very prominent and tangible result of our investigations is the online catalogue of plant species which we established and which lists extensive information about the plants we identified in two habitats of the quarry. Combined with our interactive quarry plant map, these data show which plant communities are predominantly existent on the heavy metal heap and the rough pasture. HeidelbergCement could use these multifactorial datasets to guide targeted restoration of quarry areas which will be renatured in the future. We anticipate that these plant species could be suitable for initiating faster and more efficient renaturation and, e.g., lead to easier certification in eco point assessment procedures for the company.

It is clear to us that successful restoration with ecological and societal added value will increase public acceptance of the company's quarrying activities.

2.6. Future Outlook

Our project "*Quarry biodiversity in focus – exploring the barcode of life*" is a unique collaboration between German high-school students and science educators. It has successfully combined a quarry field study with the power of DNA technologies to assess plant biodiversity.

Thorough analysis of collected plant samples has resulted in a first collection of comprehensive datasets which we deposited in a freely accessible online species catalogue. The established experimental workflow and the online data repository are openly accessible and can be used by other groups for similar investigations.

Our three-dimensional approach enabled us to combine geo location data, time and genetic information in a digital space and represents a snapshot of the biodiversity in specific habitats of the Nussloch quarry. It is important to note that species identification via our newly generated DNA barcode sequences heavily depended on the current availability of sequence data stored in the European Nucleotide Archive – a large collection of nucleotide sequences which is continuously growing. Taxonomists play a crucial role in quality assurance of deposited sequences and will continue to be the point of reference for the assignment of species vouchers to their respective DNA sequence. The datasets we generated can therefore be used in the future to re-examine the assignment to species and sub-species level.

Using the workflow established in this project, future investigations could extend our analysis by studying the two habitats over a longer period of time, comparing the distribution of plant communities during the different seasons and between years. This may allow to observe possible changes in the plant populations growing within these habitats. It could also be envisaged to subsequently expand our analysis to other quarries of HeidelbergCement that show similar characteristics. The research question could even be extended to include an investigation of the usability of these plant species as tools for remediation of soils.

A hallmark of this project is the collaboration between students and their teacher from a secondary school in Heidelberg with science educators and scientists of the EMBL. Our goal was to spark the students' interest in biodiversity-related environmental topics and to actively engage them in a real research project. The project directly raised their awareness of the importance of biodiversity management in restored mining sites and boosted their understanding of the intricate links between the industry and research sectors. The enthusiasm that was generated throughout the duration of the project is reflected in personal statements that have been provided by our project participants (see Annex).

The outcomes of this project are directly fostering the fascination for the uniqueness of quarry habitats. Based on the principle "*we can only protect what we know*", our project has further raised public awareness of the importance of biodiversity conservation to safeguard the rich ecosystems in restored quarries.

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3. Annex

- Figure 3 Map with marked locations of the two habitats within quarry Nussloch
- Table 1 Summary of plant species identified
- List of direct links to content on our project Quarry Life Award 2016 web portal:
 - o Main page: <http://emblog.embl.de/ells/qla2016>
 - o Sample collection record sheet: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-1>
 - o DNA extraction protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-2-1>
 - o PCR protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-2-2>
 - o Agarose gel electrophoresis protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-2-3>
 - o Bioinformatics analysis protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-3-1>
 - o Database search protocol: <http://emblog.embl.de/ells/qla2016/what-is-dna-barcoding/step-3-2>
 - o The European Nucleotide Archive (ENA) and other bioinformatics tools & databases: <http://emblog.embl.de/ells/qla2016/tools-and-databases/#ena>
 - o Full details of plant samples identified: <http://emblog.embl.de/ells/qla2016/plant-samples>
 - o Interactive quarry map: <http://emblog.embl.de/ells/qla2016/interactive-map>
 - o Activity reports: <http://emblog.embl.de/ells/qla2016/activity-reports>
 - o Picture galleries: <http://emblog.embl.de/ells/qla2016/activity-reports/picture-gallery>
- Personal statements of project participants

Figure 3 Map with marked locations of the two habitats within quarry Nussloch



Sample collection on the rough pasture



Students sampling the heavy metal heap habitat

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Table 1 Summary of plant species identified

Sample ID	Habitat	Location	Barcode		ENA search result / ENA accession number	Species identification	
			<i>rbcL</i>	<i>matK</i>		Scientific name	Common name (English/German)
004_C_03	HMH	49.3159, 8.7005	<i>rbcL</i>		<i>Daucus carota</i> EM_PL:DQ898156	<i>Daucus carota</i> ¹	Wild carrot Wilde Karotte
004_C_08	RP	49.3162, 8.7015	<i>rbcL</i>		<i>Daucus carota</i> EM_PL:DQ898156	<i>Daucus carota</i> ¹	Wild carrot Wilde Karotte
008_B_07	HMH	49.3158, 8.7006	<i>rbcL</i>		<i>Dianthus longicalyx</i> EM_PL:KM668208	Unclear ²	
008_B_12	RP	49.3162, 8.7018	<i>rbcL</i>		<i>Dianthus longicalyx</i> EM_PL:KM668208	<i>Dianthus seguieri</i> ³	Sequier's Pink Busch-Nelke
				<i>matK</i>	<i>Dianthus seguieri</i> EM_PL:JN589247		
008_B_33	RP	49.3162, 8.7017	<i>rbcL</i>		<i>Dianthus longicalyx</i> EM_PL:KM668208	<i>Dianthus seguieri</i> ³	Sequier's Pink Busch-Nelke
				<i>matK</i>	<i>Dianthus seguieri</i> EM_PL:JN589247		
008_B_34	HMH	49.3157, 8.7004	<i>rbcL</i>		<i>Dianthus longicalyx</i> or <i>Dianthus caryophyllus</i> EM_PL:M77699; EM_PL:KM668208	Unclear ⁴	
008_B_22	RP	49.3162, 8.7020	<i>rbcL</i>		<i>Euonymus europaeus</i> EM_PL:HE963469	<i>Euonymus europaeus</i> ¹	Spindle tree Gewöhnlicher Spindelstrauch
008_B_60	HMH	49.3159, 8.7003	<i>rbcL</i>		<i>Euphrasia</i> sp. Gaudeul 1 EM_PL:KU235128	<i>Euphrasia</i> sp. Gaudeul 1 ¹	Species of Eyebright Augentrost-Art
008_B_61	HMH	49.3161, 8.7003	<i>rbcL</i>		<i>Euphrasia</i> sp. Gaudeul 1 EM_PL:KU235128	<i>Euphrasia</i> sp. Gaudeul 1 ¹	Species of Eyebright Augentrost-Art
008_B_63	HMH	49.3158, 8.7006	<i>rbcL</i>		<i>Euphrasia</i> sp. Gaudeul 1 EM_CDS:ALX81368	<i>Euphrasia</i> sp. Gaudeul 1 ¹	Species of Eyebright Augentrost-Art
006_C_05	HMH	49.3159, 8.7004	<i>rbcL</i>		<i>Habenaria pantlingiana</i> EM_PL:KJ524104	<i>Himantoglossum hircinum</i> ³	Lizard orchid Bocks-Riemenzunge

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008_B_28	HMH	49.3157, 8.7003	<i>rbcL</i>	<i>Cardamine impatiens</i> or <i>Pachycladon ensyii</i> or <i>Pachycladon cheesemani</i> EM_PL:KJ136821; EM_PL:JX205495; EM_PL:JQ806762	<i>Lepidium campestre</i> ⁵	Field Pepperweed Feld-Kresse
			<i>matK</i>	<i>Lepidium campestre</i> EM_PL:JN894161		
008_B_32	HMH	49.3158, 8.7006	<i>rbcL</i>	<i>Lotus japonicus</i> EM_PL:AP002983	<i>Lotus japonicus</i> ¹ Synonym: <i>Lotus corniculatus</i> var. <i>japonicus</i>	Variety of the common bird's-foot trefoil Varietät des Gewöhnlichen Hornklees
008_B_35	RP	49.3162, 8.7017	<i>rbcL</i>	<i>Lotus japonicus</i> EM_PL:AP002983	<i>Lotus japonicus</i> ¹ Synonym: <i>Lotus corniculatus</i> var. <i>japonicus</i>	Variety of the common bird's-foot trefoil Varietät des Gewöhnlichen Hornklees
008_B_41	RP	49.3161, 8.7017	<i>rbcL</i>	<i>Lotus japonicus</i> EM_PL:AP002983	<i>Lotus japonicus</i> ¹ Synonym: <i>Lotus corniculatus</i> var. <i>japonicus</i>	Variety of the common bird's-foot trefoil Varietät des Gewöhnlichen Hornklees
008_B_51	RP	49.3161, 8.7021	<i>rbcL</i>	<i>Lotus corniculatus</i> or <i>Lotus japonicus</i> EM_PL:KM360864, EM_PL:HQ590168, EM_PL:AP002983	<i>Lotus japonicus</i> ¹ Synonym: <i>Lotus corniculatus</i> var. <i>japonicus</i>	Variety of the common bird's-foot trefoil Varietät des Gewöhnlichen Hornklees
008_B_54	HMH	49.3159, 8.7005	<i>rbcL</i>	<i>Lotus japonicus</i> EM_PL:AP002983	<i>Lotus japonicus</i> ¹ Synonym: <i>Lotus corniculatus</i> var. <i>japonicus</i>	Variety of the common bird's-foot trefoil Varietät des Gewöhnlichen Hornklees
008_B_08	HMH	49.3159, 8.7007	<i>rbcL</i>	<i>Rubus ulmifolius</i> or <i>Rubus caesius</i> EM_PL:FN689383, EM_PL:FN689382	<i>Rubus caesius</i> ³	European dewberry Kratzbeere
008_B_15	RP	49.3161, 8.7019	<i>rbcL</i>	<i>Rumex acetosa</i> EM_PL:KC817303	<i>Rumex acetosa</i> ¹	Common sorrel Wiesen-Sauerampfer
008_B_20	HMH	49.3158, 8.7004	<i>rbcL</i>	<i>Rumex acetosa</i> EM_PL:KC817303	<i>Rumex acetosa</i> ¹	Common sorrel Wiesen-Sauerampfer
008_B_24	RP	49.3161, 8.7018	<i>rbcL</i>	<i>Rumex acetosa</i> EM_PL:KC817303	<i>Rumex acetosa</i> ¹	Common sorrel Wiesen-Sauerampfer

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005_C_05	HMH	49.3160, 8.7005	<i>rbcL</i>	<i>Silene paradoxa</i> EM_PL:KF527887	<i>Silene paradoxa</i> ⁶	Species of <i>Silene</i> Leimkraut-Art
			<i>matK</i>	<i>Silene schwarzenbergii</i> EM_PL:FJ589557		
006_C_03	HMH	49.3158, 8.7005	<i>rbcL</i>	<i>Silene paradoxa</i> EM_PL:KF527887	<i>Silene paradoxa</i> ⁶	Species of <i>Silene</i> Leimkraut-Art
			<i>matK</i>	<i>Silene schwarzenbergii</i> EM_PL:FJ589557		
008_B_13	HMH	49.3159, 8.7005	<i>rbcL</i>	<i>Silene paradoxa</i> EM_PL:KF527887	<i>Silene paradoxa</i> ⁶	Species of <i>Silene</i> Leimkraut-Art
			<i>matK</i>	<i>Silene schwarzenbergii</i> EM_PL:FJ589557		
008_B_18	RP	49.3162, 8.7017	<i>rbcL</i>	<i>Silene paradoxa</i> EM_PL:KF527887	<i>Silene paradoxa</i> ⁶	Species of <i>Silene</i> Leimkraut-Art
			<i>matK</i>	<i>Silene schwarzenbergii</i> EM_PL:FJ589557		
008_B_57	HMH	49.3159, 8.7004	<i>matK</i>	<i>Silene schwarzenbergii</i> EM_PL:FJ589557	Unclear ⁷	
004_C07	HMH	49.3159, 8.7004	<i>rbcL</i>	<i>Verbascum thapsus</i> EM_PL:KT178130	<i>Verbascum thapsus</i> ¹	Common mullein Kleinblütige Königskerze
008_B_62	HMH	49.316, 8.7003	<i>rbcL</i>	<i>Verbascum thapsus</i> EM_PL:KT178130	<i>Verbascum thapsus</i> ¹	Common mullein Kleinblütige Königskerze
008_B_65	RP	49.3161, 8.7017	<i>rbcL</i>	<i>Verbascum thapsus</i> EM_PL:KT178130	<i>Verbascum thapsus</i> ¹	Common mullein Kleinblütige Königskerze
			<i>matK</i>	<i>Verbascum thapsus</i> EM_PL:KT176609		
008_B_01	HMH	49.3159, 8.7006	<i>rbcL</i>	<i>Veronica arvensis</i> EM_PL:KJ841651	<i>Veronica arvensis</i> ¹	Corn speedwell Feld-Ehrenpreis

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Key for Table 1:

Column "Habitat"

HMH: Heavy metal heap

RP: Rough pasture

Column "Species identification"

1: ENA search

2: Possibly *Dianthus seguieri* based on results of 008_B_12 and 008_B_33

3: ENA search, sample image

4: Sample image does not fit either species

5: ENA search, sample description

6: ENA search, within-sample *rbcL-matK* comparison of % identity

7: Possibly *Silene paradoxa* based on results of 006_C_03 and 008_B_18

Personal statements of members of the QLA project team

Supervising teacher Frank Luft

"My name is Frank Luft. I teach biotechnology, bioinformatics and chemistry at the biotechnological college (Biotechnologisches Gymnasium) of the Marie-Baum-Schule in Heidelberg. I acted as school supervisor for the project.

The students of the Biotechnologisches Gymnasium become acquainted with the molecular techniques used in this project in biotechnology lessons. The multitude of these modern methods does not allow them to be covered in their entireties in school. Working on a specific research problem and applying the theoretical study content in a practical setting is particularly beneficial for the comprehension and motivation of students. This is why it was a particular pleasure to see the great enthusiasm and fascination of the students for this project. Of course, it is something special to work in the laboratory of a world-renowned institute.

The specific topic of the Quarry Life Award was completely new to the students. Many students had little awareness of renaturation measures and the conservation of biodiversity; this is partly due to the fact that these topics are not part of the school curriculum. It was thus also a unique experience for me to see how the students developed an increased awareness and enthusiasm for these topics during the project and recognised the need and benefit of renaturation measures and the conservation of biodiversity. Developing the awareness for the need to conserve biodiversity and recognising that everyone can do something about it has, in my opinion, sparked a sensitivity for environmental sustainability in the students which is particularly significant for our society."

Student 1

"I really enjoyed the project work. I found the lab work particularly interesting. It was "something different" and it was really fun. It was a challenge to learn new things in a short period of time and to apply them, too. It worked out well and I learnt a lot. I am now better prepared for the next school year and I have also got a better idea of what I want to do after school. It was great working with Eva and Philipp. They helped us a lot and were very patient (particularly on the lab day). I am happy to have taken part in the project."

Student 2

"Working on and contributing to the project was very enjoyable. In particular, I liked the diversity of work involved: from working in the quarry and in the lab at the EMBL to the bioinformatics analysis of our sequences. Nevertheless, the project was also associated with challenges, as for myself and most other students it meant learning new things. It was difficult to comprehend all facts and processes at first, as lots of concepts were beyond of what we do in school. However, Ms. Haas and Mr. Gebhardt actively supported us, which helped us to understand the new concepts and ideas. It was this challenge that made the project so attractive and meant that we all worked with enthusiasm throughout."

Student 3

"Working on this project as part of the QLA was a unique experience. Not only did we gain insights into research practice and what it is like to work in a laboratory, but we had the opportunity to apply the theoretical approaches which we are taught in school in a practical setting. We also learnt that in scientific research things do not always work at the first try and you have to constantly deal with setbacks. I can only say positive things about our collaboration with EMBL staff and about the Quarry Life Award. I would not hesitate to take part in such a project again."

Student 4

"In May, I started working on the QLA project. Looking back and realising how many new things I learnt and how much I enjoyed it, I am extremely happy to have taken part in the project. In school, we learn a lot of theory and it was therefore particularly interesting to apply our existing and newly acquired knowledge in practice. It is something completely different to carry out an experiment without knowing the outcome (or whether there will be

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any result at all). Of course, it can also be frustrating if the hard work does not pay off and you do not get a satisfactory result at the end. But through this I experienced what research is like and that you have to be able to deal with cases like this. I am grateful I was given the opportunity to take part in this great project. A large proportion of the planning and the work was done by Philipp and Eva, who not only taught us the “know-how” but were also always available for questions. Personally, I enjoyed the exciting, but also very long and exhausting day in the lab the most. Furthermore, I also liked the bioinformatics part as this was completely new for me. I would take part in this project anytime again because you can take a lot away from it.”

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