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Aim and Scopes

Journal of Hyojeong Academia aims to serve as a global platform where researchers and scholars of various disciplines can contribute ideas for our sustainable global community of Co-existence, Co-prosperity, and Co-righteousness.

The journal is a multidisciplinary, open-access, internationally peer-reviewed academic journal, and it invites all areas of research conducted in the spirit of post materialism including studies centering on God, studies unifying religions and sciences, and studies on all aspects of Co-existence, Co-prosperity, and Co-righteousness.

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- Medical science & Medical Care
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EDITORIAL

What does the Universe consist of?

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Since the very beginning of the human history, we are striving for knowledge about the world we are living in. We don't get this knowledge intuitively, but rather use scientific method to acquire it. When we face certain feature of the world that we don't understand, we start building models of it and testing them.

So, at this state we are living in the world of models, doomed to endlessly come closer and closer to the true nature of things. Another possibility could be asking the Creator of the Universe, which is simply a one-step way, but modern science, based on the scientific method, cannot afford it.

It needs to be mentioned that some models become so habitual, that we take it for natural laws and use it in everyday life, ignorant of its incorrectness. Newton laws of motion are good examples. The first law states the existence of an inertial frame of reference (IFR), and the second law as we know it is stated to be valid in any IFR. As we do not know of any free body in our Universe, IFR can also not be constructed in it so the second law is not applicable and yet we use it in our everyday life! Why? Simply because in most of the cases the uncertainties we get due to using this wrong model are quite negligible and we need to think of more complicated models only when we deal with more accurate matters. Within this paradigm we can say that we've been through several revolutions in understanding of what does the Universe consist of, and it seems like another one is at hand!

Now we understand that any atom consists of a nucleus, containing massive protons and neutrons, and almost 2000 times less massive electrons orbiting the nucleus. Protons and neutrons are baryons, so we can say that all we see around is baryonic matter. And some might think that the whole Universe is like this - it consists of baryonic matter. How true is this?

It has been thought to be like this for a long time. We can say that all we see including planets, stars, interstellar and intergalactic gas - everything that is produced or is capable of interacting with electro-magnetic radiation and so be observed does indeed consist of baryonic matter. To describe various elements that constitute this matter we use Mendeleev's periodic table and due to modern developments in astrophysics and other fundamental sciences we now know quite well where and how did these elements come from. Soon after the Big Bang (several seconds) expansion of the Universe led to its cooling to a state when stable particles like protons, neutrons and electrons could exist and primary nucleosynthesis had occurred, but only 379,000 years later during the primary recombination epoch these could come together and form H atoms, much less of He atoms and a bit of Li atoms. Some 300 million years later these formed primordial stars, where thermonuclear reactions used these bricks to produce heavier elements up to Fe. After burning all the H fuel in thermonuclear reactions heavier stars went into supernova and even heavier elements were born in their explosions. This way simple H, He and Li multiplied into the whole periodic table. As far as we understand at the moment 88.6% of all baryonic matter atoms in the Universe are H - it is mostly intergalactic and interstellar gas, and a minor part is in stars and other massive astronomical objects. H is a main fuel for main sequence stars, so we can say that our fuel tank is almost three quarters full and we still have a long way to go as a Universe. Another piece of the baryonic pie is He - these atoms occupy 11.3% of it by number (23% mass wise) and are also present both in the interstellar medium and in stars and other bodies. All the rest of heavier atoms are within 0.1% of the pie by their numbers, which might be a surprise to a non-physicist as almost all we see around on the Earth and the Earth itself are made of these heavier atoms - a minority in our Universe!

Later in 1982 Jim Peebles assumed that existence of a substantial amount of dark matter may account for a discrepancy between the lack of essential baryonic matter density variations at the primary recombination era and the modern large-scale structure of the Universe, which could not have been able to develop in such a short

time. At the same time the theory of inflation that appeared in 1980s could explain the Cosmic Microwave Background (CMB) radiation isotropy under assumption of critical density of the Universe, which could only be reached with the existence of very significant amount of dark matter, for baryonic matter would only account for a minor part of it.

Up until now the existence of dark matter has been needed to explain not only the galactic rotation curves, dynamics and morphology of satellite galaxies and globular clusters and behavior of multi-galaxy systems. X-ray observations of hot gas in giant elliptical galaxies and clusters witness of its temperatures that are too high to be balanced only due to the visible baryonic matter gravity. Gravitational lensing allows us to see the lack of visible matter in massive galaxy clusters. Finally, some observed merging clusters witness of different plasma and main mass distributions in it. These are all hard to explain unless we assume existence of dark matter - a new type of particles, that participate in gravitational interactions, but have no connection to electromagnetism, so we cannot see it with our electromagnetic devices. We need it to be there to explain what we see, but we do not see it and do not understand what it is, therefore we call it "Dark Matter".

If only baryonic and dark matter are taken into account, equations predict a slowing down expansion, which is obvious and can be easily explained by its gravity. So it became a big surprise, when in late 1990s observations of type Ia supernova led to the opposite conclusion – the Universe seemed to be expanding with positive acceleration. It was accepted (now this issue is a point of argue) that type Ia supernova are standard candles, in other words, they should have the same luminosity wherever they appear. So, if observations show that such an object is deemer, it just means it is further and this is a good needed second procedure for measuring distance to galaxies in addition to red shift. In this research supernova were deemer than if being situated at distances predicted by the Hubble law, meaning they were further and Universe expansion is accelerated. This result was also confirmed by measurements of CMB and gravitational lensing.

The Big Bang nucleosynthesis theory gives a good explanation to formation of such light elements as De, He and Li in the young Universe. Universe large scale theory discribes well forming of stars, quasars, galaxies and galaxy clusters. Both the theories assume that baryonic and dark matter density is around 30% of the critical value. At the same time recent CMB mesurments with WMAP satellite show that global space-time curviture of the Universe is indeed close to zero, corresponding to a flat Universe and implying overall density to be around critical. But than where do the rest 70% come from?

These discripancies inspired an introduction of a new entity with negative linar coefficient in cosmological equation of state, which not only accounted for lacking energy density in the Universe, but also could be responsible for its accelerated expansion. Different observational facts agrue this entity should exist, but we do not know what it is and we do not see it directly with our electromagnetic equipment, so, as usual, let's call it Dark something! Dark Mater is taken, so let it be Dark Energy - yet another part of the Universe we cannot explain.

Since the end of inflation stage radiation played a dominant role in the evolution of the Universe. About 70,000 years from the Big Bang matter came to rule and it effected the Universe expansion. According to modern astrophysical data interpretation about 4 billion years ago the nature of expansion changed again - it started to accelerate again and we are seeing it now. Unlike Dark Matter, Dark Energy does not participate in gravitational attraction and so crowding - it is distributed homogeneously. Density of matter decreases with expansion of the Universe, but density of Dark Energy remains the same, so 4 billion years ago matter density fell below the Dark Energy density and the latter is now ruling the Universe's behavior.

So, what does the Universe consist of? Almost 70% of energy density is determined by Dark Energy, around 25% are due to Dark Matter and only 5% consist of baryonic matter. Within these 5% almost 4% represent free H and He in intergalactic and interstellar medium, 0.5% is in stars, 0.3% in neutrinos, 0.03% in heavier chemical elements and 0.01% in radiation. In other words, we know almost nothing about 95% of our home Universe and know a bit about the rest 5%.

And there is another part of our world that science will yet have to explain someday. Throughout the whole human history, the vast majority of us were believers. There are numerous reports of so-called spiritual phenomena in different parts of the world experienced through millennia of our existence. Some efforts were made to find scientific explanations of such phenomena, but they all failed. Some scientists would say that the case is closed and there is nothing to research, but other, including myself, never give up on the path of resolving worlds wonders. Imagine, if we would say: "What we see is all there is", and would not develop observational techniques

such as telescopes, optical at first, but then radio, infrared, ultraviolet, X-ray, gamma-ray, gravitational observatories – we would never know the Universe is so huge and various and consists of much more than just several thousand stars one can see in the night sky. Development of science allows us to go deeper in the nature of things in micro world as well as on the Universe-size scales. New ideas, based on deeper understanding of the physical Universe, and state of art accuracy in experiments we have achieved by today may shed a light on this terra incognita. Understanding of spiritual phenomena, based on the clear and scientific description of the spiritual world, will have to become one of the crucial goals for science in this millennium.

This is the reality of today's science and this hints us that we are living in an era on the edge of huge discoveries, when the Standard Model will have to expand to cover 5, 6 or, may be, even 7 interactions, or there may appear another model theory, describing all we know now as a whole in a completely different prospective. One thing is clear: as long as there are unresolved questions, there will always be an indestructible endeavor of human spirit to resolve it and shed the light to the unknown.

Article

Brief History of Lunar and Asteroidal Remote Sensing and Discoveries with Their Returned Samples

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Abstract: History of remote sensing studies of the Moon and asteroids changed when lunar samples were returned by the Apollo 11 mission and many meteorites were discovered on Antarctica starting in 1969. Discovery of the isotopic similarity between lunar and terrestrial materials led us to the giant-impact model to form the Moon. In addition, the existence and nature of space weathering were also discovered in 1993 by analyzing the Apollo samples. Another change occurred in 2010 when the Hayabusa spacecraft returned particles of asteroid Itokawa that proved the identity between many S-type asteroids and ordinary chondrites and the existence of space weathering similar to the Moon. The second sample return from asteroids occurred in 2020 when the Hayabusa2 spacecraft returned samples of C-type asteroid Ryugu. In spite of some expectations, it was a pristine CI1 chondrite material that was free from terrestrial contaminations suffered by known CI1 chondrite meteorites. Sample return missions drastically improved the accuracy of our knowledge on the raw materials of solar system planets and will surely keep revealing the secrets behind the birth of this special planet Earth. This part of history also teaches us that scientists should proclaim the truth against denial or persecution by others.

Keywords: remote sensing, sample return, Moon, asteroids, Apollo 11, Hayabusa, Hayabusa2, OSIRIS-REx

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1. Introduction

Our very existence highly relies on the special characteristics of planet Earth, and discovering the origin and evolution of the Earth requires studying the Solar System as a whole, which falls into the field of planetary science. Planetary science has been mostly developed through remote sensing of planets and satellites by ground-based, airborne, and space telescopes, and spacecraft missions. Atmospheric compositions of planets are relatively easy to investigate through such remote sensing. However, in order to fully understand the reason why our Earth-Moon system has its current configuration including the sizes, dynamics, and compositions, we really need to study solid samples of planetary bodies. For that purpose, meteorites and interplanetary dust particles (IDPs) were in the past the only such samples available of solid planetary materials although they were somewhat altered by the terrestrial environment. In 1969, the Apollo 11 mission returned the first lunar rocks and regolith materials as the first recovery of pristine extraterrestrial solid materials, the Japanese Antarctic Research Expedition (JARE) discovered nine distinct meteorites concentrated at the foot of Yamato Mountain Range [1], and two large carbonaceous chondrites (CCs), Murchison and Allende, fell in Australia and Mexico, respectively [2]. These events allowed researchers to examine many detailed aspects of solar system formation such as elemental, isotopic, and mineral

abundances, physical characteristics such as density and porosity, and alteration such as heating, shock, and space weathering. In this brief paper, the history of lunar and asteroidal remote sensing and discoveries from their returned samples are reviewed.

2. Moon

Throughout history, the Moon has been the target of imagination, observation, studies, and exploration. However, the origin and evolution of the Moon had not been clear until after Apollo missions returned samples in 1969-1972, which led researchers to form and confirm the giant-impact hypothesis for forming the Moon [3].

2.1. Lunar remote sensing

Galileo Galilei started observing and sketching the Moon in 1609 [4]. He provided not only evidence that the lunar surface is rough but also how it could be derived even from naked-eye observation that the entire sun-lit surface is illuminated instead of just a longitudinal line which would specularly reflect sunlight if the surface were totally smooth [5]. This was an example of thought experiments he also used in concluding both heavy and light objects fall at the same speed [6]. Albert Einstein also employed thought experiments in deriving special and general relativity theories. Geniuses like them could obtain much more truth from limited facts than ordinary people.

Since then, there were many telescopic observations and spacecraft missions to study the Moon before the first crewed landing by the Apollo 11 mission. While many researchers were lost in identifying the surface composition of the Moon, Bruce Hapke correctly predicted the presence of silicate minerals and space weathering by simulating solar wind with H^+ ions irradiated on basalt powders that altered their photometric, polarimetric, and spectroscopic properties to resemble those of the lunar surface [7, 8]. Unfortunately, his idea of space weathering was not well accepted by the science community at that time, and he redirected his research efforts toward developing a spectrophometric model, which is now well-known as Hapke's model [9]. A bad thing can indeed end up being a good thing.

2.2. Moon-forming hypotheses

There were four main hypotheses for how the Moon was formed [10]:

- a) Capture: The Moon was captured by the Earth's gravity as it passed nearby.
- b) Accretion: The Moon was created along with Earth at its formation.
- c) Fission: The Earth had been spinning so fast that some material broke away to form the Moon.
- d) Giant impact: A Mars-size planet hit a proto-Earth, and the debris from this impact formed the Moon.

The accretion and fission hypotheses could not easily explain the difference between the Earth's spin axis and the Moon's orbital axis, the capture hypothesis could result in the Earth and the Moon having totally different compositions, while the giant-impact hypothesis predicted high compositional similarity between the Earth and the Moon. Therefore, finding the compositional difference or similarity between the Earth and the Moon was the key to narrow down the origin of the Moon between the capture and the giant impact hypotheses. Remote sensing alone could not achieve this goal because it would take isotopic analysis of solid materials to validate the common origin of two planetary bodies.

2.3. Apollo sample analyses

When the samples returned by the Apollo 11 and subsequent missions were analyzed, their oxygen isotopic compositions were identical to terrestrial materials within analytical errors [11], which added support to the giant-impact hypothesis. In addition, when a high-voltage transmission electron microscope (TEM) was utilized to examine sub-microscopic-scale composition of Apollo lunar soil particles, nanophase metallic iron ($npFe^0$) particles were found within a thin (100-200 nm) amorphous vapor coating layer [12], confirming the existence of space weathering as Bruce Hapke predicted [8]. The lunar surface became darker and redder through space weathering by solar wind implantation and micrometeorite bombardments over a long period of time. Discovering the products of space weathering ($npFe^0$) took 26 years after Apollo 11 samples were returned since the analytical techniques had to be developed.

This is an excellent example of the fact that sample return missions allow discoveries by not only the present-day scientists and their techniques but also the future more knowledgeable scientists with more advanced techniques.

3. Asteroids

Around the same time when Apollo 11 samples were first analyzed, asteroid 4 Vesta was spectroscopically identified as similar to a basaltic achondrite meteorite [13]. This was a confirmation that meteorites could be samples of asteroids and gave hope to identify their parent bodies among asteroids through reflectance spectroscopy. However, very few more pairs of asteroids and meteorites turned out to show spectral matches.

3.1. S-type asteroids and ordinary chondrite controversy

As shown in Fig. 1 (a), asteroid 4 Vesta had been the only asteroid whose reflectance spectrum matched with meteorites for a long time since 1970. The main problem was the spectral mismatch between the most common ordinary chondrite meteorites and the most abundant S-type asteroids in the inner main belt. Ordinary chondrites include the H, L, and LL classes in the order of high to low iron content, and the spectral mismatch is demonstrated in Fig. 1 (a) with the Näs LL6 chondrite (black solid line) and asteroid 7 Iris (red filled squares) as examples. If the lunar-type space weathering is occurring on the S-type asteroids, ordinary chondrites could change their spectra to the S-asteroid spectra. However, there had been no npFe^0 particles or any other type of clear space weathering products discovered in our ordinary chondrite meteorite collections, although, a npFe^0 -rich rim was found the Kapoeta howardite meteorite [14] which likely came from a V-type asteroid as possible evidence of space weathering. Therefore, almost no meteoriticist had believed in asteroidal space weathering.

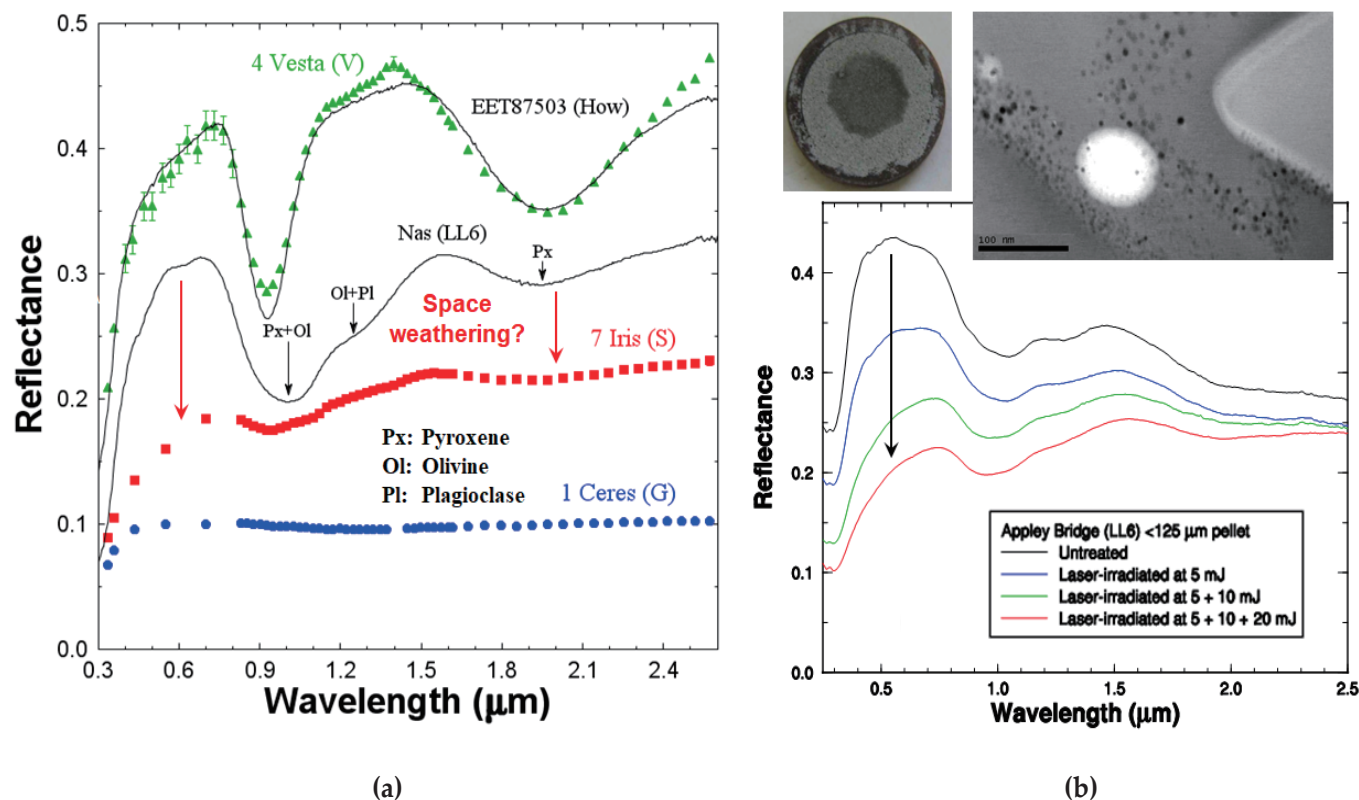


Figure 1. (a) Comparison of visible to near-infrared reflectance spectra of asteroids and meteorites, where telescopic asteroid spectra are shown in colored markers, and laboratory meteorite spectra are shown in black solid lines, and asteroid and meteorite classes are indicated in parentheses. Note that 1 Ceres is now classified as a dwarf planet; (b) Pulse-laser irradiation on a powder pellet sample of Appley Bridge LL6 chondrite meteorite at various accumulated amounts of energy.

3.2. Pulse-laser irradiation experiments to simulate space weathering

In 1999, a successful space-weathering simulation was performed using pulse-laser irradiation of pressed powder pellet samples of olivine and pyroxene (which are the main mineral components of ordinary chondrites) in vacuum [15]. As shown in Fig. 1b, as the laser energy was increased, the ordinary chondrite spectrum became darker and redder to resemble those of S-type asteroids, and npFe⁰ particles were discovered in the amorphous vapor coating layer as in the case of Apollo soil particles. This was the first proof that ordinary chondrites could change their reflectance spectra into the S-type asteroid spectra in the similar space environment to that of the Moon in terms of having no atmosphere and residing in the inner solar system. Using these artificially space-weathered olivine and pyroxene spectra, two asteroid spectra were nearly perfectly fit to demonstrate the existence of space weathering on those asteroids [16].

However, in the same manner as happened to Bruce Hapke's H⁺ ion implantation experiments, many researchers either neglected or attacked the above results by Japanese scientists, even to the level of rejecting the author's abstract submitted to the 30th Lunar and Planetary Science Conference (LPSC) in 1999 with obviously nonsense reasons such as "the wavelength of the pulse laser was not written" because it was common knowledge that YAG laser had a wavelength of 1064 nm.

3.3. Hayabusa mission returned samples of S-type asteroid 25143 Itokawa

In 2005, the Japanese Hayabusa spacecraft rendezvoused with S-type asteroid Itokawa [17], and despite having many accidents and challenges, miraculously returned its sample-laden capsule to the Earth in 2010. During the rendezvous phase, the Near-Infrared Spectrometer (NIRS) onboard the Hayabusa spacecraft measured reflectance spectra (0.75-2.1 μm) on many spots of Itokawa's surface [18].

Shown in Fig. 2 are modified Gaussian model (MGM) [19] fittings of four representative spots, and Gaussian band center and relative band strength values of a larger number of spots, plotted along with those of ordinary chondrites including the Hamlet LL4 chondrite pellet irradiated with a pulse laser. In Fig. 2 (a), it is clear that all four spectra share almost the same Gaussian band center values with different band strengths and continuum background spectra (broken curves). The continuum slopes indicate increasing degrees of space weathering in the order of Western Bright Area (blue filled triangles), MUSES-C Regio (green filled squares), and Ohsumi Basin (red filled circles). The close-up spectrum (black asterisk) close to the MUSES-C Regio spectrum (red filled square) corresponds to about a 1 cm size footprint, which signifies that the surface is highly homogenous down to the cm scale, consistent with ordinary chondrite lithology. In Fig. 2 (b), it is evident that Itokawa spectra (black filled circles) are most similar to those of LL chondrites (red open circles) and more spectra are similar to laser-irradiated LL chondrite spots (red filled circles).

These results indicates that Itokawa is made of LL-chondrite materials and its surface is space-weathered in various degrees, consistent with another study employing Hapke's space-weathering model [20]. Analyses of mostly tiny (~0.1 mm) particles returned from Itokawa revealed these rendezvous phase remote-sensing results were correct [21, 22].

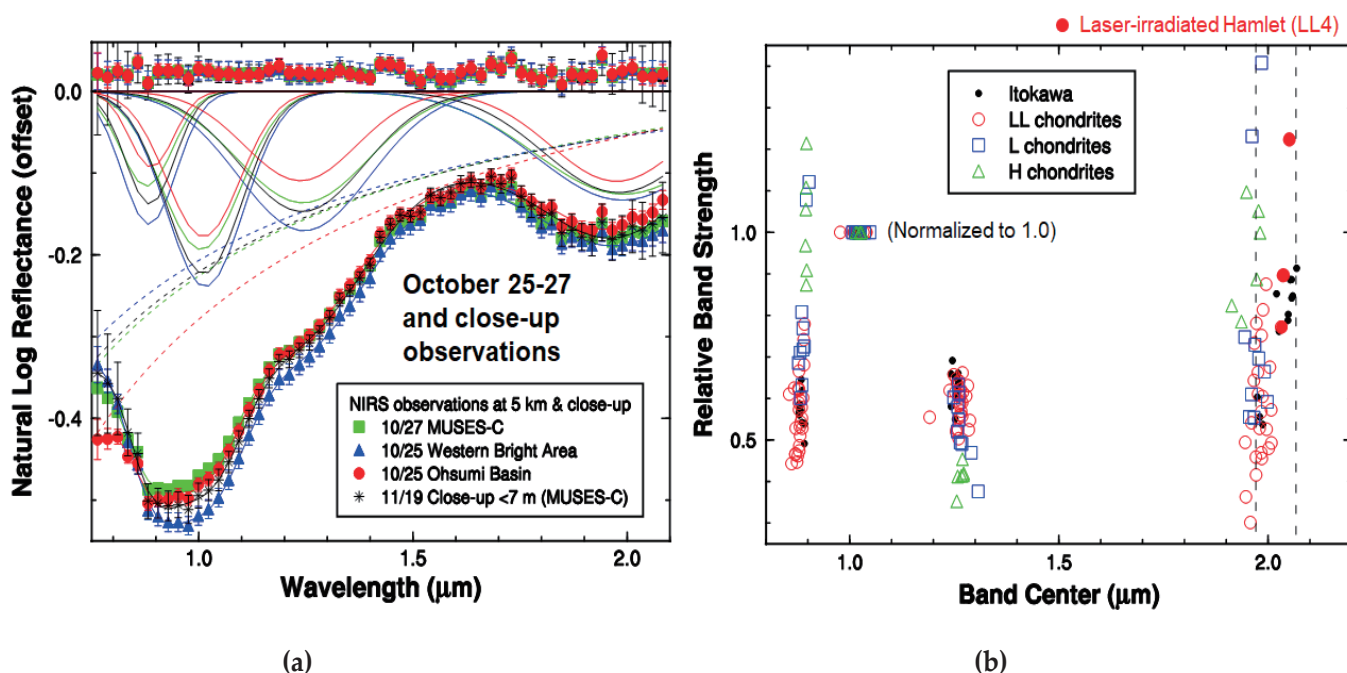


Figure 2. (a) Modified Gaussian model (MGM) fittings of four select reflectance spectra of asteroid Itokawa spots taken by the Near-Infrared Spectrometer (NIRS) onboard the Hayabusa spacecraft; (b) Gaussian band center vs relative band strength of a larger number of Itokawa spectra (black filled circles) plotted along with those of ordinary (H, L, and LL) chondrites including a Hamlet LL4 chondrite pellet irradiated with pulse laser (red filled circles).

3.4. Hayabusa2 mission returned samples of C-type asteroid 162173 Ryugu

In 2019, the Japanese Hayabusa2 spacecraft landed twice on C-type asteroid Ryugu, and returned its samples to Earth in 2020. During the rendezvous phase, the Near-Infrared Spectrometer (NIRS3) onboard the Hayabusa2 spacecraft measured reflectance spectra (1.8-3.2 μm) of the surface spots to detect hydration features near 2.7 μm , indicating that Ryugu's surface was made of partially dehydrated CI chondrite or shocked CM chondrite material [23]. However, returned Ryugu samples (5.4 g in total) turned out to be pure CI chondrite materials, totally free of terrestrial contamination, heating, or significant shock [24]. Also, by comparing the 2.7 μm hydroxyl absorption band position between the returned sample and the NIRS3 data of Ryugu, a 6 nm shift was detected that is evidence of space weathering by solar wind [25].

The reason why the remote-sensing based prediction was incorrect is that the unheated CI chondrite samples in our collections were all contaminated with terrestrial materials and altered (oxidized) by the Earth's atmosphere, forming secondary minerals. We simply never knew what the true, pure CI chondrite spectrum should look like. This situation is illustrated in Fig. 3. Alais CI1 chondrite in our collection shows a much brighter spectrum with features such as UV and broad 3 μm band which are absent in Ryugu sample spectra. On the other hand, Ivuna CI1 chondrite heated at 500°C shows a much closer spectrum to Ryugu spectra. The probable explanation is that CI1 chondrites in our collection contain terrestrial contamination including iron hydroxides, and moderate heating them in vacuum could remove them although too much heating would totally dehydrate saponite and serpentine, erasing the 2.7 μm band. Samples of another C-type asteroid 101955 Bennu were returned by NASA OSIRIS-REx mission, and their detailed analyses are now being undertaken to find any similarity or difference from Ryugu samples.

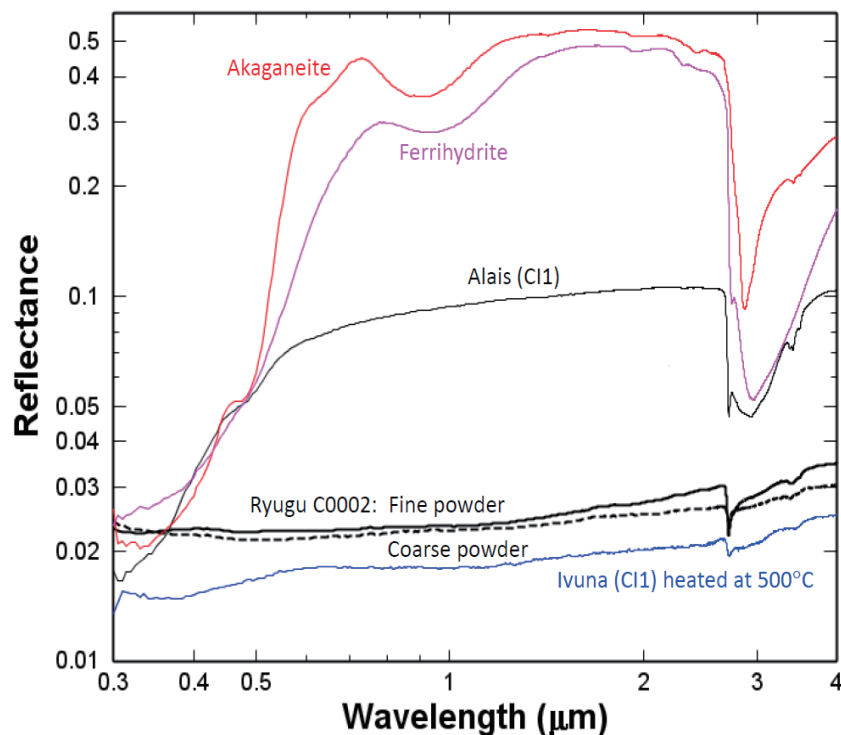


Figure 3. Visible to near-infrared reflectance spectra (0.3-4 μm) of fine and coarse powder samples of asteroid Ryugu [24], powder samples of Ivuna (CI1) meteorite heated at 500°C, of Alais (CI1) meteorite, ferrihydrite, and akaganeite, which are all taken from the RELAB database [26].

4. Summary

Lunar sample returns by the Apollo missions revealed the origin of the Moon and the existence of space weathering of airless bodies. S-type asteroid Itokawa sample return by the Hayabusa mission proved that space weathering altered asteroid surfaces with ordinary chondrites hidden among S-type asteroids, and the Hayabusa2 mission provided a lesson and a reminder that our CI chondrites (and undoubtedly others) are terrestrially contaminated or altered. In order to correctly integrate our knowledge of the Moon, asteroids, and lunar and meteorite samples, space weathering and terrestrial weathering must be evaluated and removed. Along with expanding remote-sensing studies, sample return missions should give us trustable key elements for revealing the secrets of the birth of the special planet Earth where intellectual life emerged and developed science. Another important lesson is that even the world greatest scientists are imperfect, having prejudice against different opinions, etc. We must be obedient to the facts and humbly pursue the truth with unbiased reasoning and imaginations.

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Article

Comparative genomic study of polar bacteria having tolerance to abiotic stress and potential for environmental and agricultural implementation

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Abstract: The polar regions are home to a variety of microorganisms. These microorganisms have been reported to have developed adaptive methods for survival in extreme conditions and resist varieties of abiotic stress such as heavy metals (HMs). Despite this, very limited studies have been done in bacteria from polar regions than bacteria from non-polar regions. The main aim of this study is to explore microorganisms from polar regions that could tolerate the abiotic stress of HMs. In this study, microorganisms from polar areas have been isolated and various bioinformatics tools were used for understanding the genomic features, comparison, and analysis. The wet-lab experiments were performed for the validation where the isolated bacteria were exposed to the abiotic stress of HMs. The genome analysis of all the isolated bacteria showed the presence of heavy metals resistance proteins. This study is very helpful in exploring the diversity of abiotic stress resistance microorganisms, monitoring environmental health, and utilizing these potential microorganisms for the betterment of the environment, agriculture, and ultimately humankind.

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1. Introduction

The *Sphingomonas* genus was defined by Yabuuchi et al., in 1990 and is a member of the family *Sphingomonadales*, phylum *Pseudomonadota*, and class Alphaproteobacteria [1]. They have been reported to be isolated from the soil, water, air, and marine environment [2-5]. In addition to that, many strains that exhibit the ability to use the toxins as nutrition have been identified from environments contaminated with hazardous chemicals. Furthermore, *Sphingomonas* are gram-negative, aerobic, rod-shaped, chemoheterotrophic bacteria that produce yellow or off-white pigmented colonies and possess a characteristic compound called sphingolipids. The sphingolipids have a unique sphingoglycolipid with the long-chain base dihydro sphingosin, ubiquinone 10 (Q-10), and 2-hydroxymyristic acid (2-OH C14:0) and the absence of 3-hydroxy fatty acids [6, 7]. Both pathogenic and non-pathogenic *sphingomonas* have been reported. *Sphingomonas elodea* strains (PHP1 and PBAD1) were reported as non-pathogenic [8] and *Sphingomonas meloni*, as phytopathogenic which is also linked to the demise of coral reefs off the coast of Florida [9, 10]. *Sphingomonas* species were well reported to degrade PAHs including fluorene and fluoranthene [11, 12]. They are HMs/metalloid-tolerant, with the potential to be used in bioremediation purposes [13, 14].

The *Mesorhizobium* genus was identified by Jarvis et al., in 1997 [15] is a member of the family *Phyllobacteriaceae*, phylum *Pseudomonadota*, and class *Alphaproteobacteria* [16, 17], and have been reported to be isolated from the seawater, wastewater treatment system, and groundwater [18–20]. They are also gram-negative, aerobic. Bacteria like *Mesorhizobium* but they are non-spore forming. The broad distribution of this genus and their ability to make a symbiotic relationship to several plant genera makes them an interesting candidate for agronomic and ecological applications [21, 22]. The majority of research on HMS in *Mesorhizobium* has been conducted in polluted and agricultural environment [23, 24]. The majority of *Mesorhizobium* research is focused on symbiotic relationships, plant growth promotion, nitrogen fixation in plants like chickpeas, and resistance ability to various antibiotics [25–29]. Besides that, *Mesorhizobium* species were also reported to have tolerance to heavy metals as well as potential for bioremediation of heavy metals [30, 31]. Even though both *Sphingomonas* and *Mesorhizobium* were reported to have HMs tolerance capacity and are well-known in contaminated and agricultural settings, very little work has been done from the polar areas.

Polar regions, being an isolated environment and having an extremely harsh climate have been reported to be affected by increased human activities including climate change and global warming. Furthermore, the abiotic stress of HMs has been reported in the polar areas. The microorganisms living in these areas have been reported to have developed adaptive strategies to survive in extreme conditions and resist varieties of abiotic stresses [32]. The development of these strategies in polar microorganisms is a fascinating area of research. Despite this fact, very limited studies have been done on bacteria from the polar areas of distant locations and harsh climates. In addition to that, the information regarding the diversity of such microorganisms is very limited.

The main objective of this study was to isolate such types of bacteria from various polar areas and perform genomic analysis and wet-lab experiments. In this study, we have isolated three different bacteria from the polar region two bacteria of the same genus but different species and one bacteria of a different genus. Genomic analysis was done by using various bioinformatics tools to understand all the genomic features and wet-lab experiments were performed. All the isolated bacteria might have the potential to be utilized in the future for the betterment of the environment, agriculture, and ultimately humankind.

2. Materials and Methods

2.1. Isolation and genomic DNA extraction of polar bacteria to study their adaptability to tolerate abiotic stress, as well as the study's possible application to future environmental and agricultural aspects

Sphingomonas sp. (1) and *Sphingomonas* sp. (2) were isolated from a rock of an arctic lichen *Umbilicaria* sp. and an arctic lichen *Cetraria* sp. respectively whereas, the strain *Mesorhizobium* sp. was isolated from soil samples from Uganda. All three bacteria were culture for 4-5 passages in an R2A medium. Then the bacterial strains were sub-culture in R2A agar plate 2-3 times at 15°C on R2A agar until the pure single colony of each strains were obtained. After that, each individual colony of bacterial strains were cultured and genomic DNA from all the bacteria was extracted using the QIAamp DNA Mini Kit, and its quantity and purity were assessed using a spectrophotometer. The extracted DNA was further evaluated for quality using agarose gel electrophoresis and stored at -20°C.

2.2. Genome sequencing and assembly process

The genome sequencing for *Mesohizobium* sp. was carried out using the PacBio RS II single-molecule real-time (SMRT) sequencing technology from Pacific Biosciences (Menlo Park, CA, USA). SMRTbell library inserts of 20 kb were prepared and sequenced using SMRT cells. The raw sequencing data were generated and subjected to de novo assembly utilizing the hierarchical genome assembly process (HGAP) protocol [33] and RS HGAP4 Assembly in SMRT analysis software (ver. 2.3; Pacific Biosciences, SMRT Link 4.0.0) protocols. The annotation of the genome was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Furthermore, coding DNA sequences (CDSs) were predicted and annotated using the Rapid Annotation using Subsystem Technology (RAST) server [34]. The genome sequencing of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) was analyzed by using a combined approach with the 454 GS FLX Titanium system (Roche Diagnostics, Brandford, CT) with an 8-kb paired-end library and the illumina GAIIx system (San diego, CA) with a 500-bp paired-end

library The detailed about these strains were mentioned by Jungeun Lee et al., 2012 [35] and Hyoungeok Lee et al., 2012 [36].

2.3. Functional annotation and comparative genomics analysis

Annotation tools such as RAST server was used, predicted gene sequences were translated and subjected to the National Center for Biotechnology Information (NCBI) non-redundant database, UniProtKB/Swiss-Prot, and Protein Data Bank proteins (PDB). The comprehensive annotation approach allowed us to gather a thorough understanding of the genomic features of all the strain (*Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp.

2.4. Bacterial isolation and growth

The *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. was isolated using 0.1 x R2A agar (MB cell Ltd., Seoul, Korea). The environmental temperature during the isolation of the strain was 15°C.

2.5. Bacterial tolerance test to heavy metals

The tolerance of all three bacterial strains towards the heavy metals (HMs) was measured at 15°C by using salts of heavy metals such as copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, and 0.01 mM. Spectrophotometer was used for the measurement of bacterial OD_{600} . All the experiments were performed in triplicates.

3. Results

3.1. Genomic features and genome analysis

Sphingomonas sp. (1) and *Sphingomonas* sp. (2) are draft genome and *Mesorhizobium* sp. is a complete genome. The genome analysis of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) showed the presence of copper resistance protein (CopB, CopC, and CopD) and cobalt-zinc-cadmium resistance protein (CzcC and CzcD). And *Mesorhizobium* sp. showed the presence of copper resistance protein (CopC and CopD) and cobalt-zinc-cadmium resistant protein (CzcD).

3.2. Growth of the bacteria

All bacteria, *Sphingomonas* sp. (1), *Sphingomonas* sp.(2), and *Mesorhizobium* sp. was cultured in R2A agar plates at 15°C (Fig. 1).

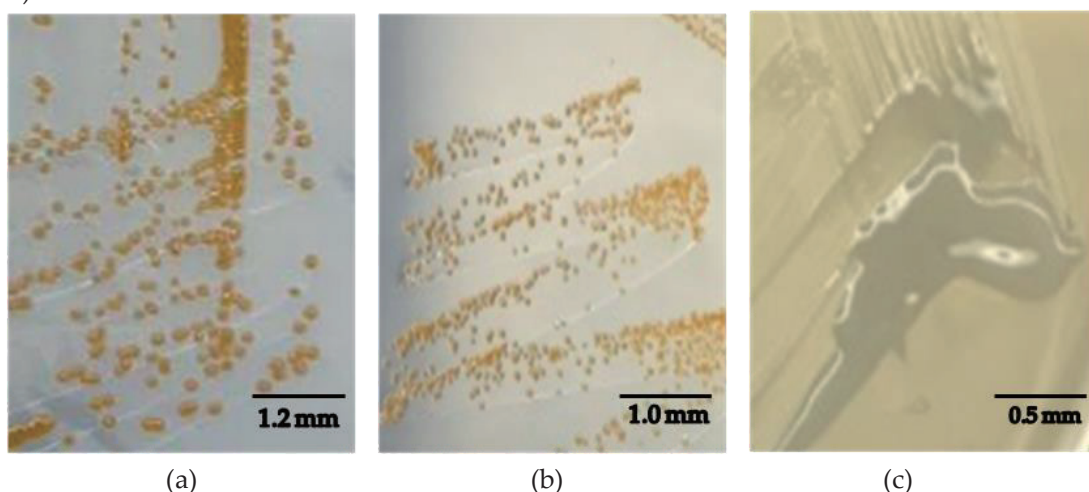


Figure 1. (a) *Sphingomonas* sp. (1); (b) *Sphingomonas* sp. (2); (c) *Mesorhizobium* sp.

3.3. Tolerance of bacteria to different heavy metals (HMs)

Three strains *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. showed HMs resistance protein from their genome analysis. Since all the strains showed copper resistant protein and cobalt resistance protein so

all the strains were subjected to HMs resistance toxicity tolerance assay using the salts of copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$). The concentration of copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) used were 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, and 0.01 mM. Among the three strains, *Sphingomonas* sp. (1) showed tolerance to 0.1 mM of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.25 mM of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, *Sphingomonas* sp. (2) showed tolerance to 0.1 mM of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and *Mesorhizobium* sp. showed tolerance to 1 mM of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 2 mM of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ respectively at 15°C. Overall, these results indicate that the three bacterial strains have varying degrees of tolerance to the tested heavy metals at the given temperature. Among the three bacterial strains tested, *Mesorhizobium* has the highest tolerance capacity for both the metals copper as well as cobalt.

Table 1. Table showing HMs toxicity tolerance by *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp.

(Used concentration of HMs)	<i>Sphingomonas</i> sp. (1)		<i>Sphingomonas</i> sp. (2)		<i>Mesorhizobium</i> sp.	
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
2 mM	-	-	-	-	-	+
1 mM	-	-	-	-	+	+
0.5 mM	-	-	-	-	+	+
0.25 mM	-	+	-	-	+	+
0.1 mM	+	+	+	+	+	+
0.05 mM	+	+	+	+	+	+
0.01 mM	+	+	+	+	+	+

4. Discussion

HMs pollution had become an urgent issue worldwide which need rapid solution for future sustainability. Moreover, HMs pollution affected the polar regions too and the community of microbes living there. Microorganisms living there have been reported to have developed adaptive strategies. Furthermore, Microbes were reported to confer various types of resistance mechanism in response to HMs [37]. Copper resistance protein such as (CopA, CopB, CopC and CopD) were reported in various bacteria. These protein were responsible for copper resistance and maintenance of homeostasis [38-40]. Furthermore, multiple proteins such as CzcA, CzcC, and CzcD responsible for resistance of cobalt, zinc, and cadmium were also reported in various bacteria [41, 42]. The comparative study of genome analysis of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) showed the presence of copper resistance protein (CopB, CopD) and cobalt-zinc-cadmium resistance protein (CzcC and CzcD) in both the strain. The comparison between *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) in terms of HMs, the former strain showed tolerance of 0.25 mM of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and the latter strain showed tolerance of 0.1 mM of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. In the case of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, both strains showed tolerance to 0.1 mM of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ at 15°C. *Mesorhizobium* sp. showed the presence of copper resistance protein (CopC and CopD) and cobalt-zinc-cadmium resistant protein (CzcD). *Mesorhizobium* sp. were able to tolerate up to 1 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 2 mM of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ at 15 °C. Even though our isolated strain did not showed very high tolerance but showed some tolerance to HMs than the other microorganism from polluted areas, the fact that the polar areas are not exposed to high pollution and considered as pristine [43] should be noted.

5. Conclusions

The polar regions are home to many different microorganisms even being isolated environment with extreme climate. Information regarding the diversity of such microorganisms is valuable. Besides that, these microorganisms have developed various strategies to cope with extreme environment and tolerance to stresses like HMs. In this study, we compare three bacteria isolated from the polar region (*Sphingomonas* sp. (1), *Sphingomonas* sp. (2),

and *Mesorhizobium* sp.) in terms of their genomic features. Furthermore, these strains showed HMs tolerance at low temperature. These strains might have potential for bioremediation in future. This study is very helpful to find the diversity of abiotic stress (HMs) resistant microorganisms, to monitor environmental health, and to utilize these potential microorganisms in future for the betterment of the environment, agriculture, and ultimately humankind.

6. Summary

Three different bacterial strains namely, *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. were isolated from polar regions. All three bacterial strains showed HMs resistance protein (copper resistance and cobalt resistance protein) and were able to tolerate HMs at 15°C.

Author Contributions: T.-J.O. designed and supervised the project. A.K. performed the experiments; A.K., S.-R.H., H.L., and T.-J.O. wrote the manuscript. All authors discussed the results, commented on the manuscript, and approved the manuscript.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Insight into the therapeutic potential of Antarctic mosses: An untargeted metabolomics approach

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Abstract: Over the course of history, plant materials have been utilized for healing various ailments. The knowledge derived from traditional medicine has proven invaluable, serving as a foundational resource for drug discovery and design. However, the journey from screening natural products to the development of drugs is a prolonged and demanding process. Fortunately, with the emergence of sophisticated analytical techniques and the establishment of global databases containing information on thousands of natural products, the screening and discovery of bioactive compounds from complex sample mixtures have become more time-efficient and less labor-intensive. In particular, the non-targeted metabolomics approach proves advantageous, offering a comprehensive analysis of a wide range of compounds, aiding in the detection of both known and unknown substances. This approach is especially beneficial for gaining a thorough understanding of the pattern of metabolites found in Arctic and Antarctic vegetation, among the least explored areas on Earth. Here, we employed a non-targeted metabolomics approach to analyze the chemical components in Antarctic mosses. While many of the compounds could not be predicted, some were identified as therapeutic agents, with a few exhibiting pharmacological properties such as anticancer, anti-inflammatory, antilipemic, anti-diabetic, anti-adipogenic, and neuroprotective effects. Notably, we predicted that one moss sample produced desulpiram, an FDA-approved drug for the treatment of chronic alcoholism, and methyl palmitate, well-known for its anti-inflammatory potency, could be detected in many of the samples. Furthermore, we experimentally verified the therapeutic potential of Antarctic moss extracts by conducting a cyclooxygenase-2 (COX-2) inhibition assay, assessing their potent anti-inflammatory activity. Indeed, two of the moss samples exhibited significant COX-2 inhibitory potency. Hence, our findings emphasize the advantage of metabolomics in providing insight into the potential of Antarctic moss as a valuable natural resource for the discovery and development of potent therapeutics. Additional research is due in order to identify and define the particular bioactive compounds accountable for the observed effects.

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Keywords: Antarctic mosses, non-targeted metabolomics, metabolites, pharmacological properties, COX-2 inhibition.

1. Introduction

Indigenous knowledge, traditional medicine, and ethnopharmacology have forged a rich foundation for the discovery of therapeutic agents [1]. The wealth of information embedded in the wisdom of diverse cultures and centuries-old practices, often rooted in the use of local flora for medicinal purposes, has set the stage for unlocking the latent potential of nature's pharmacy [2, 3]. Even though the use of natural products (NPs) for medicinal purposes dates back to 2600 BC [4], the chemical space of undiscovered NPs still outweighs that of those already known [5, 6]. This setback could be due to high cost, time consuming and labor-intensive applied methods [7]. However, recent advancements in

computing and information processing keep us hopeful as we can systematically explore uncharted territories for potential drug candidates by harnessing the power of high-throughput screening processes [8]. The rapid analysis of vast datasets across available databases of natural products will expedite the identification of promising compounds from untapped resources [9].

Metabolomics refers to the comprehensive measurement of all metabolites and low-molecular-weight molecules in a biological specimen. And metabolic profiling is the systematic analysis, identification, and quantification of metabolites within a specific biological system [10]. Both processes are valuable for understanding the complex metabolic processes and their respective metabolites. Based on the approach two types of metabolomics can be applied, targeted and non-targeted. As the name suggests, non-targeted metabolomics encompasses the global measurement of all metabolites in a sample, including both known and unknown targets [11]. Utilizing analytical techniques such as mass spectrometry, non-targeted metabolomics offers high-throughput screening of compounds [12].

For example, untargeted metabolomics has been extensively used in the study of traditional Chinese medicine (TCM) for purposes ranging from identifying bioactive compounds [13] to evaluating toxicology [14]. Additionally, by integrating metabolomics with network pharmacology, researchers have proposed optimized formulations of TCM for treating various systemic diseases [15, 16]. These examples clearly demonstrate that untargeted metabolomics is a key trend in natural product research, drug discovery, and development.

Integrating metabolomics for the exploration of Antarctic mosses highlights the importance of this captivating frontier in drug discovery. These resilient organisms, thriving in one of the harshest environments on Earth, hold the promise of unique bioactive compounds (anti-freeze proteins, UV-protective substances, or novel antibiotics) [17]. By applying an untargeted metabolomics approach, we can delve into the intricate and unique biochemistry of Antarctic mosses, unraveling their therapeutic potential. The vast natural product database, cultivated through the amalgamation of traditional knowledge and computational screening, serves as a reservoir of information to guide this exploration.

Therefore, this study carried out the exploration of the less studied resource Antarctic mosses leveraging analytical techniques, computational tools and database searching (metabolomics) to unravel their therapeutic potential. Furthermore, experimental evaluation was performed where the moss extracts were assessed for their bioactivity using COX-2, an enzyme involved in inflammatory response in the body [18]. The significance of the COX-2 inhibition assay lies in its ability to identify compounds that can potentially act as anti-inflammatory agents, which is crucial for developing new treatments for inflammatory diseases.

2. Materials and Methods

2.1. Chemicals

Solvents used for extraction such as methanol and acetone were purchased from Daejung Reagents Chemicals (>99.8%) and Samchun Pure Chemicals (99.7%) respectively. HPLC grade solvents were used for Ultra-High Performance Liquid Chromatography (UHPLC) bought from Fisher Scientific, Korea. A COX ovine/human Inhibitor Screening Assay Kit 560131 (Cayman Chemical, Ann Arbor, MI, USA) was used.

2.2. Moss sample processing and extraction

Moss samples were obtained from the Polar Natural Product Chemistry Laboratory of the Korea Polar Research Institute, here labeled as MS-1, MS-3, MS-6, MS-9, MS-10, and MS-13. Each samples were extracted using two organic solvents: acetone and methanol. Processing involved grounding of the dried samples in a mortar, weighing about 3 grams of the powdered sample and distribution into each flasks. The flasks were filled with (200 mL) acetone and extracted at dark and room temperature condition for 24 hours with constant stirring. The mixture was filtered, and the filtrate extracts dried under reduced pressure. The process was repeated thrice and after the third extraction, the solvent was replaced by methanol and the process continued as before. In this way, acetone and methanol extracts for each moss samples were obtained. The dried crude extracts were dissolved in HPLC grade methanol for further analysis.

2.3. Untargeted metabolomics

2.3.1. UHPLC analysis

Preliminary analysis of metabolites was conducted on a Shimadzu Nexera UHPLC system equipped with a Shim-pack GIS C18 column (4.6 X 250 mm, particle size 5 μ m HSS) connected to a Photo Diode Array (PDA) Detector. The mobile phase consisted of two solvent system, solvent A consisting of water and solvent B, acetonitrile. The elution method followed a gradient flow starting off at 5% solvent B reaching 100% at 28 min. The flow rate was maintained at 1.0 mL/min.

2.3.2. LC-MS/MS analysis

A Thermo Scientific UHPLC, Ultimate 3,000 RSLC System coupled to a Q-Exacte Plus Orbitrap mass spectrometer was used for untargeted LC-MS/MS. Chromatographic separation in the LC system carried out using Acquity UPLC BEH C18 column (2.1 X 100 mm, 1.7 μ m) with two solvents water (C) and acetonitrile (D), both acidified with 0.1% formic acid. The gradient elution method was as follows: 5% D (2 min), 5-100% D (2-9min), 100% D (9-13min), 100-5% D (13-13.1 min), and 5% D (13-16min). the column temperature was maintained at 50°C, injection volume of 5 μ L and flow rate of 0.4 mL/min. Mass spectra were recorded in full MS-ddMS2 positive mode at the range of 80-1000 m/z. The operation parameters were as follows: collision-induced dissociation energy, 30 V; resolution, 70,000 for full MS and 17500 for ddMS2; ion spray voltage, 3.5 kV; and capillary temperature, 370°C. The Raw MS data files were searched against databases such as High-Resolution Accurate Mass (HRAM), MassList, ChemSpider, etc. and matched spectrums annotated.

2.4. Raw data processing and molecular network building

Further analysis of the raw data was performed as described previously [19,20] with some modifications. Briefly, the raw files were converted into a suitable "mzML" format. For molecular network building, Global Natural Product Social (GNPS) (<https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp>) molecular networking platform was utilized. The converted files were uploaded into the GNPS site through WinSCP software. Another platform, SNAP-MS (www.npatlas.org/discover/snapms), processed the network generated from GNPS, annotating the subnetworks. The resulted files were downloaded as a graphML network file and visualized in Cytoscape (<http://cytoscape.org/>).

2.5. COX-2 inhibition assay

In-vitro COX-2 inhibition assay was carried out using COX (ovine/human) Inhibitor Screening Assay kit from Cayman Chemical following the instruction in the manual provided. The tested concentrations were 50 and 500 μ g/mL. Positive control contained only DMSO. For negative control, the enzyme was subjected to thermal denaturation. Data were represented as the percentage of control.

2.6. Statistical analysis

Experiments were performed in triplicate and the results were expressed in terms of mean \pm standard deviation.

3. Results

3.1. Preliminary analysis of metabolites

Reversed phase HPLC of the crude extracts of Antarctic mosses showed multiple peaks in the chromatogram indicating the presence of diverse range of compounds with varying polarity. Figure 1 shows chromatogram of MS-10 and MS-13 acetone and methanol extracts with solvent blank as methanol. Indicated by red arrows, distinct peaks may be unique components of the extracts. In comparison to MS-13 methanol and acetone extracts, MS-10 methanol extracts contained relatively polar compounds as shown by the early elution time ($rt < 6.0$ minutes) of the peaks which indicated that moss samples contained distinct compounds with differing chemical properties.

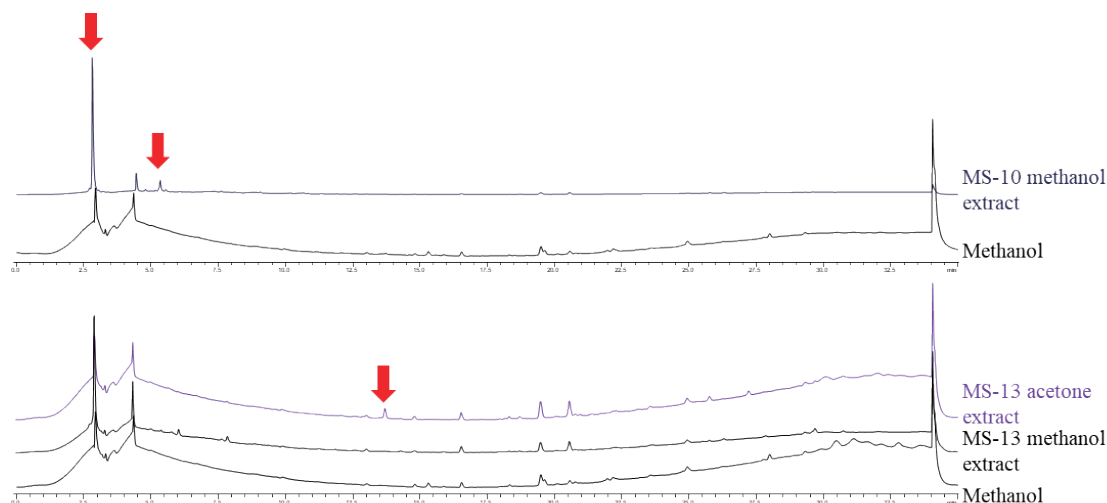


Figure 1. HPLC chromatogram of methanol and acetone extracts of MS-10 and MS-13 samples with distinct peaks indicated by red arrows.

3.2. Untargeted metabolomics

3.2.1. LC-MS/MS analysis

For a more comprehensive exploration of the metabolic profiles of the crude extracts, LC-MS/MS was performed. Database searching of the obtained MS/MS data aided in compound annotation. Indeed, some of the predicted compounds were known for their therapeutic properties, whereas few were FDA approved drugs. For example, disulfiram and meglutol were among the predicted compounds that are prescribed for treatment of alcohol addiction and as an antilipemic agent respectively. Table 1 lists the common annotated compounds predicted from MS-10 and MS-13 with their bioactivity.

Table 1. List of some compounds present in Antarctic mosses crude extracts and their features identified using LC-MS/MS analysis including their therapeutic properties (reference studies)

S. N.	Compound name	RT (min)	Molecular formula	Exact mass	Therapeutic potential
1	Choline alfoscerate	0.669	C ₈ H ₂₀ NO ₆ P	257.1023	Neuroprotective agent [21, 22]
2	Disulfiram	0.624	C ₁₀ H ₂₀ N ₂ S ₄	296.0501	Treatment of alcohol use disorder [23]
3	D-Raffinose	0.594	C ₁₈ H ₃₂ O ₁₆	504.1683	Anti-adipogenesis, anti-diabetic [24]
4	Meglutol	0.604	C ₆ H ₁₀ O ₅	162.0526	HMG-CoA inhibitor, antilipemic agent [25]
5	Methyl palmitate	9.781	C ₁₇ H ₃₄ O ₂	287.2818	Anti-inflammatory, anti-fibrotic effect [26]
6	Oleamide	9.813	C ₁₈ H ₃₅ NO	281.2713	Treatment of sleep disorder, neuropharmacological activity [27, 28]
7	Stigmasterol	12.986	C ₂₉ H ₄₈ O	412.3697	Anti-diabetic, anti-cancer [29, 30]

3.2.2. Molecular networking analysis

Molecular networking is used to analyze unknown compounds by grouping compounds based on their fragmentation spectra similarity. Figure 2 represents the collective molecular network profile of the acetone and methanol extracts of MS-10 and MS-13. Distinct clusters of compounds were observed possibly representing either different classes or family of compounds which requires further exploration.

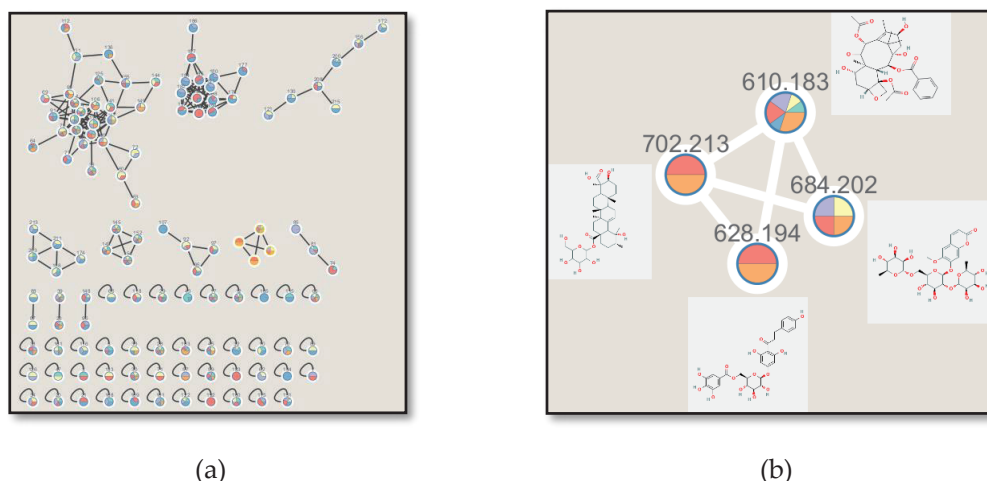


Figure 2. Molecular networking of Antarctic mosses based on LC-MS/MS: (a) Clusters of networks representing compounds with similar fragmentation spectra; (b) Cluster highlighted in yellow indicating predicted compounds sharing similar structures.

3.3. COX-2 inhibition activity

COX-2 activity for each sample treatment was expressed as percentage value with respect to the 100% activity control (Figure 3). COX-2 activity was lowest for MS-13 acetone and MS-10 methanol extracts suggesting presence of COX-2 inhibitors. This underscores the potential of Antarctic moss samples, specifically MS-10 and MS-13, in the development of natural product-based anti-inflammatory treatments.

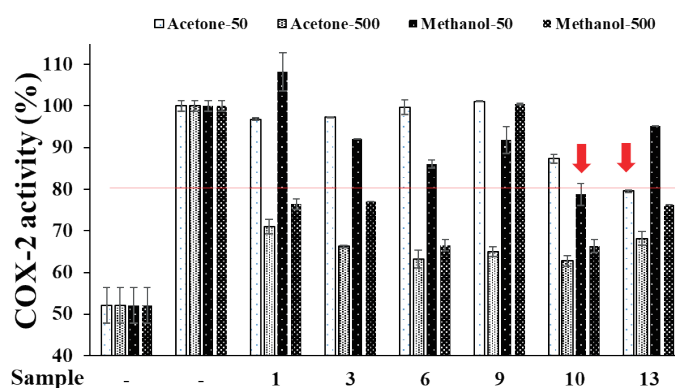


Figure 3. Inhibitory effect of acetone and methanol extracts of different moss samples on COX-2 enzyme activity.

4. Discussion

The increasing need for potent therapeutics in response to various health challenges underscores the significance of investigating underexplored sources such as Antarctic mosses. Utilizing compound databases constructed through extensive research, traditional medicine knowledge, and computational tools, it is now more efficient and relatively simpler to explore the chemical space of these resources, aiding in drug discovery and development [8, 31].

Metabolomics, specifically untargeted metabolomics, aims to characterize and categorize metabolites or compounds present in complex samples like crude extracts by comparing their mass spectral and/or NMR data with established reference databases. Through this approach, this study enabled us to explore the therapeutic potential of Antarctic mosses. Using solvents with differing polarity, acetone and methanol, compounds varying in chemical properties could be obtained as observed in the chromatogram suggesting that the Antarctic moss samples differed in their chemical compositions. Indeed, subsequent LC-MS/MS analysis and chemical compound annotation found that each sample extract contained compounds that were unique to the moss sample (data not shown). Additionally, compounds with known therapeutic potential including some FDA approved drugs were

identified within the Antarctic moss samples. However, the list only encompassed a fraction of the known compounds. A large portion of the extracts contained numerous unknown compounds, prompting the utilization of computational tools capable of clustering compounds based on similarities in their fragmentation patterns. This facilitated the creation of network structures representing known and unknown compounds, offering initial insights into the structural properties of the unknown compounds. Following experimental validation revealed that certain samples (MS-10 methanol extract and MS-13 acetone extract) exhibited COX-2 activity inhibition, indicating their potential therapeutic efficacy. Overall, the findings suggest that among the six Antarctic mosses, MS-10 and MS-13 contained bioactive compounds, specifically potent COX-2 inhibitors, implying their potential use in anti-inflammatory therapeutics.

The implications of these findings extend to addressing global health challenges and mitigating the imbalance between health risks and available therapeutics. The pharmaceutical industry faces significant hurdles in drug development, and exploring natural products from Antarctic mosses holds promise for discovering unique compounds that could contribute to the drug discovery process.

However, the study primarily serves as a preliminary screening method for bioactive compounds. While Antarctic mosses demonstrate potential for uncovering natural treatments for various diseases, more advanced techniques are necessary for identifying and discovering novel compounds. Future research efforts will involve isolating and establishing structure-activity relationships (SAR) of specific bioactive compounds to enhance our understanding of their mechanisms of action.

5. Conclusions

Traditional knowledge and advancements are the two sides of a coin. In the realm of medicinal research, integrating both aspects can unravel new avenues for therapeutic exploration. Through a comprehensive metabolomics approach (the knowledge and advancement), this study has shed light on the potential of Antarctic mosses (the unknown) as a source of bioactive compounds (the acknowledged) with promising therapeutic applications. By bridging traditional knowledge with modern analytical techniques, it became possible to uncover a rich reservoir of metabolites within these resilient plants, offering a glimpse into their therapeutic potential. Our findings underscore the importance of interdisciplinary approaches in drug discovery, where the convergence of traditional wisdom and scientific innovation can lead to novel therapeutic interventions. As we navigate the challenges of addressing evolving health concerns, exploring the untapped resources of our natural world, such as Antarctic mosses, holds great promise for the development of future pharmaceuticals. It is our hope that this study serves as a catalyst for further research, inspiring collaboration between traditional knowledge holders and scientific communities to unlock nature's pharmacopeia and improve global health outcomes.

Finally, it is important to remember that nature has interconnected various species by endowing mutual benefits, enabling them to address each other's challenges; thus, uncovering therapeutic significance and preservation of these vital ecosystems from extinction amid the changing environment ought to go hand in hand towards maintaining a sustainable environment.

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Conflicts of Interest: The authors declare no conflict of interest.

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Article

Ownership of Health Data as the new Frontier for the Future of VODAN-Africa

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Abstract: The Covid19 situation created a state of unequal use of data to produce vaccines. Africa was not in control over sovereign decision-making relating to the health of its citizens and to access of the data pertaining to it. The available digital architecture in Africa health facilities allowed data to be siphoned to Europe and the US at will. The purpose of the research is to argue why medical data once created should be held in ownership of the facility where the data is produced under the regulatory framework of the county and national state jurisdiction. This is referred as data that is Findable, Accessible (under well-defined conditions) Interoperable and Reusable (FAIR). The study inventories the additional benefits of FAIR data for health purposes as means to assist Africa generate (self-)employment benefits to many unemployed youths based on the value that data holds in the digital economy. The study was carried out using a case study approach with four health facilities in Kenya, to help identify the contribution of the FAIR data concept to producing a sustainable outcome for health data management in Kenya. The paper discussed the potential challenges which should be overcome to realize the full potential of FAIR data for a sovereign management of the health data in Kenya. Health data remained as an asset for the health facility for providing improved health at point of care. The findings show digital health data has an economic value and health benefit if well managed at point of production.

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Keywords: Digital architecture, FAIR Data asset, improved health and sustainable outcomes

1. Introduction

The state of the post Covid-19 pandemic in Africa calls for new paradigms of thinking around data ownership. Data ownership is now an emerging key contributor to country specific economic development and sustainability. The report from World Health Organization [1] estimated that global spending on health was reaching US \$8.3 trillion between 2020 and 2018, which is about ten percent of global GDP. It underlines why there is a growing need for an organized system of health data collection and storage. Research by Royal Bank of Canada [2] shows that data generated by the health care industry is growing faster than manufacturing, financial services, media and the entertainment sector. Thirty percent of the world's data is generated by the healthcare industry and during the Covid-19 pandemic the demand for data continued to grow [2]. The RBC research teams [2] and Financial Times at the same time revealed that Big Tech companies have seen an opportunity to harness the ever-growing amount of information in advance from various

countries to champion and pioneer new innovations and light up the health care revolution. RBC research calculated that by June 2021, the venture capital divisions of Amazon, Apple, Facebook, Google and Microsoft had invested more than \$7 billion in healthcare start-ups. Africa Private Equity and Venture Association [3] (AVCA, 2021) agrees with the RBC statistics by adding that funding African start-ups is growing fast with venture capital deals reaching \$3.5 billion by mid-2022 [3].

This is to challenge the commercial imperatives and charitable efforts where vaccines continue being the exclusive control of private companies through intellectual property and manufacturing capacity monopolies, resulting in fatal vaccine inequity [4]. The fierce competition by wealthy countries to buy up the vaccines even before they were product buy up all vaccines and deny poor countries access can be avoided when respective countries of Africa control their own health data and agree to enter strong equal partnerships with the rich nations and private donors.

To make a point for this future investment agreement, the IMF has evaluated existing agreements focusing on 2025 where US \$50 billion has been put aside from donors and national governments to strengthen existing mechanisms, with attention put on ACT-A and including vaccine purchase and distribution facility COVAX, which could generate US \$9 trillion of additional global output. IMF estimates that sixty percent (60%) of gains would benefit developing countries, especially Africa [5].

The World Health Organization (2020) report on Global Vaccine Market Report reiterates the lack of resilience and limited geographic spread to manufacturing infrastructure before the pandemic where ninety percent (90%) of all vaccine production was concentrated with four companies, GSK, Pfizer, Merck and Sanofi. In terms of volume the Serum Institute of India was the lone largest producer with 28% of estimated 5.5 billion vaccines produced by 2020 [6, 7].

The demand for vaccine manufacturing capacity for Covid-19 vaccines has shown limitations to global manufacturing infrastructure, with relatively little vaccine capacity able to produce at large scale outside of major vaccine corporations and SII, and a high dependency on a very limited number of producers [8]. WHO (2021) reports that 99% of its vaccines are imported. The United Operation Warp Speed alone has invested over US \$10 billion in the R&D of six promising vaccines and US \$2.5 billion for Moderna and US \$1.5 billion for Johnson & Johnson [9]. The German Government invested US \$450 million to BioNTech [10, 11]. WHO (2021) reports that 97% of financing to develop the AstraZeneca/Oxford Vaccine came from public funds including the European Union and Governments of United Kingdom and Northern Ireland [1, 9, 12, 13, 14, 15]. Mazzucato et al [16] have criticized this approach and suggested COVID-19 patent rights should be mutually binding among collaborating nations.

In another study by Salient Advisory, cited by Thabiso Foto [17], states that while the pandemic itself had a negative effect, healthcare infrastructure development has come out as a significant opportunity, for ensuring equitable access to quality healthcare among all the 54 countries of Africa. The pandemic created this necessity for accelerated development of healthcare solutions by tech entrepreneurs. In another study by Salient Advisory, cited by Thabiso Foto, states that while the pandemic itself had a negative effect on ordinary health of millions of people, Africa is home to 1,276 health tech start-ups that are supporting health care delivery and distribution. By 2020 over 60% health tech start-ups had already been founded in Africa. In 2020 alone over 22% of the companies were founded as the pandemic surged. The question arising from this data is who owns and controls this sensitive health data. Once tech start-ups take control of data there is the big privacy question and security challenge to individual health data. Therefore, African governments can find worthy investors in their own data production and data infrastructure systems if they have ownership over the data. With available data, Africa will be able to produce its own vaccines and compete among emerging global players in vaccine production alongside China, Russia to help diversity the global manufacturing.

Hence, health data are a critical resource for development of products in health. Peter Clardy, the senior clinical advisor at Google, was able to demonstrate that patient data infrastructure development would have a comparative advantage when data is collected and curated at respective country specific health facilities. The East African Health Research Commission [18] had started working on digital health innovation policy by 2017 by acknowledging the potential of patient data as Findable, Accessible (under well-defined conditions, Interoperable and Reusable (FAIR) [19]. The Virus Outbreak Data Network (VODAN)-Africa research reveals that countries of

Africa have developed capacity and technological advancement in the development of health data infrastructure to make sure data is in residence of its collection point, within health facilities where patients are treated from.

Vodan Africa was founded by leading researchers in Africa in 2020 at the height of covid 19 pandemic. The goal was the use of data as a value contribution to containing and treatment of covid 19 pandemic. The leading universities in Africa included Mekelle University and Addis Ababa University (Ethiopia), Kampala International University (Uganda), Tangaza University (Kenya), Olabisi Onabanajo University (Nigeria), Ibrahim Badamasi Babangida University Lapai (Nigeria) University of Sousse (Tunisia), Great Zimbabwe University and East African University, Mogadishu, (Somalia). Today, 2024, VODAN now translates to value driven ownership of data and accessibility network. The East African Health Research Commission fell short of incorporating a VODAN-Africa type of infrastructure to make their data FAIR. The potential of a FAIR-based data infrastructure for Kenya is investigated in this paper. This is referred as data that is Findable, Accessible (under well-defined conditions) Interoperable and Reusable (FAIR). GO-FAIR Leiden refer to Wilkinson by describing FAIR data principles as the contribution of information to gaining access to that data and making it compatible with other data with the aim of reusing it [19].

1.1. *The limitation of the study*

The study looked generally on the past experiences of losing data collected during the Ebola endemic and felt the same situation should not happen again during the covid-19 pandemic. The selected countries as points of reference to data ownership, were seven in total from Africa namely Ethiopia, Kenya, Somalia, Tanzania, Zimbabwe, Nigeria and Tunisia. Selecting health facilities from these countries was more of a demonstrative sample of the challenges Africa was facing compared to developed nations of the west. In 2020, UN Secretary General, Antonio Guterres, while addressing the UN Security Council on equitable distribution of vaccines. He fell short of suggesting that while the G20 would form the task force, Africa too should be well represented in the task force. He had observed that while, "The rollout of COVID-19 vaccines is generating hope," the 15-member council chaired by Kenya then, the injustice was in vaccine equity. He is quoted saying, "At this critical moment, vaccine equity is the biggest moral test before the global community." It implies that Africa should not be reduced to a perpetual emergency only continent without structures in place to speak, discuss, compliment and ask for the necessary resources to produce and own its own vaccines [20].

Therefore, the selected seven countries and the four health facilities in Kenya may not fully give specific details of challenges in the 54 countries in Africa. However, the discussion helps the reader to develop a general overview on the situation of four health facilities in Kenya cuts across the experience of losing data to the west in Africa.

1.2. *Statement of Contribution*

VODAN-Africa has identified a gap in the digital architecture development in Africa. It makes a strong point that while Africa has been open to improving its digital health infrastructure, most donors and sponsors for advanced technology do not respect basic interests for data ownership in the continent. Most of the digital health data collected is easily siphoned to Europe and the US, thus creating a situation in which Africa is not in control over sovereign decision-making relating to the health of its citizens. The unjust and poor centralized control of data has denied subjects access to data as a resource for information, knowledge and development.

VODAN-Africa is making a strong case for health data to be controlled by the government under the Ministry of Health and curate who accesses this data. To do so, requires an organized and updated health data infrastructure system to realize the full potential of Findable, Accessible, Interoperable and Reusable (FAIR)-data for a sovereign management of the health data in Kenya.

This article explores the potential for advanced new methodologies for enhancing health data as a public good that can contribute to improved health outcomes in Africa, and how such potential can be unlocked to contribute to the development potential of the continent. The study was carried out using a case study approach with four health facilities in Kenya, to help identify the contribution of the FAIR-data concept to producing a sustainable outcome for health data management in Kenya, while also discussing the potential challenges that may need to be overcome to realize the full potential of FAIR-data for a sovereign management of the health data in Kenya.

The paper concludes by showing why digital innovations, are key to data collection and storage but also creating room for economic advantage unlike the past when data could be siphoned out to the developed world.

The main question is an investigation of the current data handling and value of the health data in Kenya to demonstrate how VODAN-Africa is offering an alternative architecture for handling health data by using four health facilities in Kenya as case studies, with extended data produced across 88 health facilities across Africa. The rationale is that advanced FAIR-based systems for data collection, storage and curation within the facilities under the Ministry of Health can contribute to data sovereignty, ethical data curation for the production of valuable data, and the creation of job opportunities for a sound economic development in Africa in the digital era.

To answer the research question, the paper discusses the key problem area, a methodology to investigate the current data handling and value of the data, the health data policies in Kenya. The study then discusses the implementation of an alternative architecture in four health facilities by VODAN-Africa.

2. Materials and Methods

The research included (i) a desk study analysis of the current health data infrastructure in Kenya; (ii) an investigation of an implementation of an architecture to curate patient data as FAIR. The desk study was carried out in a systematic desk review involving all relevant policy statements of the Government of Kenya. The study referred journal papers from WHO and policy statements by the Kenya Government on health data and infrastructure development. The paper identifies policy directions proposed to realize the full potential of data for a better management of the health data in Kenya. The different government policy papers around FAIR facets constituting core principles showed equivalency with the ambition of the Kenyan government, and it demonstrated the available potential in making sure health data is secure at locality of access and under the arm of the Ministry of Health as discussed in Ester Thea Inau et al [21].

In order to carry out the second purpose of this study, case study in four health facilities in Kenya was carried out. These were (1) Zambezi Hospital (private hospital) and (2) Pumwani Hospital (under Nairobi County Ministry of Health) and (3) Beacon of Hope (private an in Kajiado County). (4) Kenya Medical Research Institute was also involved in the study to assess the outcomes. The research followed a case study approach implementing the innovation in a natural setting. The assessment included participation of the various stakeholders and took an interdisciplinary approach involving all stakeholders, including the ICT technical teams, electronic and computer network engineers, medical doctors, nurses, social scientists, policy makers, social workers and health data stewards.

3. Results

3.1. Economic benefits of health data

The data presentation and discussion from the four health facilities in Kenya, reveal the importance of data for the economic benefits of each country in Africa. While we see the importance of health data for economic development, we need to review what Africa needs to do to take the full value of its health data. Each country can curate the data for its own economic development and job creation especially in ICT technology, especially in digital health innovations [10].

The Council on the Economics of Health for all comprised economists and experts in health under WHO [11] laid a clear vision on how to develop a new understanding and a new narrative about the deep interconnectedness between health and the economy [22, 23]. The vision focus of WHO Economics Health Council was to give direction on intertwined core themes for countries to adopt as follows:

1. There has to be measurement and valuing of Health for All.
2. This calls for capacity strengthening of the public sector in building resilient capacities and creating partnerships to deliver health for all.
3. A key strategic support pillar is finance. This is core to strategic, long term, and transformative finance for all. It calls for
4. Governing innovation towards health for all and very importantly improving the governance of innovation, which has been identified as the critical building block of healthy economies.

5. The governance of health innovation ecosystem lays a good foundation upon which radical changes are needed to ensure it delivers Health for All [12].
6. The long-term vision of WHO Economics Health Council is to offer guidelines on the establishment of new, end-to end health innovation ecosystem that shapes how the public and private sector in health delivery can work together throughout the innovation chain to deliver equitable access to much needed vaccines, therapeutics, diagnostics and other important health supplies
7. The health innovation ecosystem should be governed towards the common good of all by creating purpose driven innovation through a mission-oriented approach. It means reshaping knowledge governance for the common good, reforming corporate governance to better reflect stakeholder value in the long term.
8. This Council also sets goals for building resilience and diverse manufacturing capacity and infrastructure development as now done by VODAN-Africa.
9. Lastly, the call is for introducing conditionality for public investments to build symbiotic public private partnerships, and strengthening the capacity of the public sector in health innovation.

The WHO Economics Health Council gives a global vision on how countries can move towards health data economic benefits by allowing multiple actors and investors in this sector. It also gives guidelines to governance issues so far ignored by private tech start up innovators. Health innovation ecosystem should be governed towards the common good of all within specific countries where health data is generated from Yamey et al [13]. Above all the WHO Economics Health Council agrees with the direction that VODAN-Africa has taken on developing digital architecture to allow Africa governments to be in control over sovereign decision-making relating to the health and allow its citizens access the data that pertains to its health subjects as a resource for information, knowledge and development.

The entry of VODAN-Africa into the space of data infrastructure will develop the much-needed capacity within each country to collect, store and curate much needed data to induce investment into manufacturing of vaccines. The World Health Organization also identifies knowledge barrier as an area which continues to stifle innovation, especially when it comes to the availability and use of vital health technologies and data. Knowledge generation and sharing are critical for medical research and public health is limited. The current system incentivizes innovation through monopolies, in which governments allow the privatization of biomedical knowledge through granting patent protections is not adequate. There is need for availability and use of vital health technologies and data.

WHO called for redesigning health ecosystem for the common good, by shifting from a model where innovation is seen to be driven by market forces, to a model that is collectively governed in public interest. It means each country should embrace innovations that support all aspects of healthcare. To achieve this requires proper tools for health data collection and local storage. This the key contribution by VODAN-Africa. With the entry of VODAN-Africa data research teams, sharing data on medical research and public health. African countries with the support of African Union are challenged to join hands and set aside funds for more data research and data infrastructure development.

3.2. Health data as a valuable resource

VODAN-Africa ventured into understanding the demand for COVID-19 data and its local availability, the major gap was data infrastructure development. The lack of interoperability and reuse of data in parallel health structures was weakening the value of digital data health solutions. The commercial use of that data will create distrust without a sound data collection point in the health facilities. This is why VODAN-Africa understands the purpose of having different regulatory frameworks for data capture and handling with a renewed vision on how to handle data analysis in the continent compared to others.

The opportunity generated by ownership of health data opens a new frontier for the future of Africa. Just as the past centuries the world went through different economic development models that dominated the exchange of merchandise and capital goods using gold and silver, today data production and storage is taking over as the 'new gold' in terms of its importance to being the source for economic development. In our case then, data production and storage in the local country, within health facilities, is starting to influence a new form of economic

value which could benefit not only nations without any minerals but also become a beacon for offering job opportunities in the ICT sector in developing countries of Africa. Taking for example, the recent Covid-19 pandemic data, which is well curated will contribute to vaccine production and stimulate marketing opportunities for nations where Covid-19 pandemic was dominant.

The importance of having up to date machine infrastructure for data production and storage has stood out as an important entry gap for VODAN-Africa. The nine African countries that participated in the maiden research in digital health can attest to the fact that many opportunities have arisen for well-trained data stewards, health records officers and put demand to hospital administration to develop good health infrastructure systems which can keep this important data under lock and key within the country and in health facilities. Instead of relying on data collected and sent abroad with less access by patients and administration, a window of opportunity beacons for African countries to compete and be the custodians of their own data.

3.3. The difference between health tech start-ups and innovation by VODAN Africa

The outbreak of the COVID-19 pandemic made it necessary for countries to look into the whole digital architecture as far as data management was concerned. Until 2020 most health data in Africa was siphoned and funneled to Europe and the US. One good example was when in 2016 Ebola virus was discovered in West Africa and parts of Central Africa, many well intending agencies from the West came into collect data and treat victims of Ebola infection. After six months the data could not be traced in countries where the Ebola virus had struck.

With the emergence of the coronavirus pandemic, most countries of Africa were cautious about keeping data locally for new vaccines development and improving quality of health. The creation of VODAN-Africa in 2020 was to give an answer to the much-awaited clinical data production with concerns for data ownership and improving on what had been until 2020, a limited use of health data for quality treatment at point of care [14].

Most African countries had weak data infrastructure system for storage and curation at point of production, especially in health facilities. Most countries in Africa had foreign owned data infrastructure. The District Health Information System is installed and used by most countries of Africa without the knowledge that this application is foreign owned. It means any patient data collected may not be kept locally but banked abroad. From this data collected it was possible to develop vaccines and sell at exorbitant prices to the very countries they siphoned data from.

It is evident that African countries were not in control over sovereign decision-making process relating to the health data. Their citizens cannot access the data that pertain to resource for information, knowledge and development. VODAN-Africa developed an architecture to record clinical health data and research data collected on the incidence of the COVID-19 pandemic, by producing human and machine-readable data. This architecture supports analytics at point of care through data (re)-visiting, across health facilities, for generic analytics. Algorithms are run across FAIR Data Points to visit the distributed data and produce aggregate findings, based on permissions obtained prior to the analysis. The FAIR data architecture was deployed in Uganda, Ethiopia, Liberia, Nigeria, Kenya, Somalia, Tanzania, Zimbabwe, and Tunisia. It was evident that different contexts should be explored so that the problem at hand could be properly defined and responded to [15].

3.4. Data control policy in Kenya

The Kenya Constitution and Vision 2030 development blueprint requires the country to provide the highest attainable standard of healthcare through adoption and use of ICT and the Ministry of Health developed the Kenya Health Policy fulfil this mandate [24]. This comes at an important time when the health sector is implementing far reaching reforms to achieve universal coverage. This concept is anchored on the achievement of Vision 2030, whose overall goal in health is to have an “equitable and affordable healthcare at the highest achievable standard” to her citizens. It is informed by the strategies and results emanating from the implementation of the Kenya Health Policy Framework, 1994-2010, the health sector strategic plans and the e- Government and Shared Services Strategies implemented through the e-Government Directorate and the ICT Board respectively.

In the past thirty years the Kenya government has come to understand the value of developing policies that would address the importance of data generation with FAIR pertaining to the qualities of findability, accessibility, interoperability and reuse. However, the gap remained how to make sure that data is controlled by the government through respective ministry of health and local health facilities.

The solution of VODAN-Africa was that data is created and held in the facility where the data is produced under the regulatory framework of the county and national state jurisdiction. This delivers a resource in which the data remains as an asset for the health facility, both in terms of providing improved health care as well as contributing to other use of the data. The discussion features the economic aspect where the data is produced in Africa to generate (self-)employment and benefits in Africa.

The Kenya Health Policy, 2014-2030 gives directions to ensure significant improvement in overall status of health in Kenya in line with the Constitution of Kenya 2010, the country's long term development agenda, Vision 2030 and global commitments. It demonstrates the health sector's commitment, under the government's stewardship, to ensuring that the country attains the highest possible standards of health, in a manner responsive to the needs of the population. This policy is designed to be comprehensive and focuses on the two key obligations of health: realization of fundamental human rights including the right to health as enshrined in the Constitution of Kenya 2010 and; contribution to economic development as envisioned in Vision 2030 [24].

However, to achieve this milestone, the Kenya Government set up an authority to monitor and direct all data information and especially health data. The Kenya ICT Authority was created in 2019 to lay out a platform for better policy implementation and monitoring. Under this authority then, it is understood that the specific mandate is to ensure coherence and a unified approach on the principles that govern the acquisition, deployment, management and operation of data production in ICTs across the public service, state agencies, with the aim of promoting service integration, adaptability, and cost savings through economies of scales in ICT investment. Compliance to this state policy will bring about an efficient and effective service delivery to citizens (GOK, 2016, Kenya Health Enterprise Architecture, ICT Authority Government ICT standards, first edition 2016) [25].

The Kenyan policy lays a good foundation for making sure data is stored in Kenya for better use and support to wellbeing of the country. However, the weak area not address with this ICT Authority mandate is the discussion of FAIR data and curation. It remains vague on how health data can be stored, controlled and shared, for research while allowing any independent agency to access, control and use patient data. The examples in mind are the tech start-ups which are not under an organized government health data infrastructure. VODAN-Africa has found a way of helping respective governments especially Kenya Government ICT Authority to solve this problem.

3.5. Case study of four health facilities

In 2020 the COVID-19 pandemic affected the whole world. A group of researchers involving nine countries of Africa came together to address data from COVID-19 for future use and storage in local health facilities. The study teams from these countries looked at the existing data infrastructure systems in each country. They commonly used the district health information science. Countries like Kenya later adopted DHIS2 to Kenya Health information system. But the key gap remained the same. Working within the framework of the health ACT (2017, revised in 2019, 2021) the Health Act Section XV, article 104, directs the Ministry of Health to administer health information banks, including an interoperability framework, data interchange and security [26]. The VODAN-Africa Kenya team led by Tangaza University College, Nairobi, supported by the VODAN-Africa technical teams in Mekelle University, Leiden University Medical Centre, and Stanford University, were able to understand why data interoperability is not enough. The whole data infrastructure required a big overhaul. There was need to develop software to allow a new application which would test how data can be collected, stored and curated within local health facilities and remain available and interoperable with data held in other facilities elsewhere. Therefore, the VODAN-Africa built a platform which comprises a network of systems fitted with sets of tools to produce, use and reuse FAIR data. This system builds on the globally known and widely used standards that allow FAIR Data management. By the time the project was finished in 2022, 88 health facilities had been accessed included and 77 were producing data in more than nine countries across Africa.

The Figure 1 demonstrates the process followed by VODAN-Africa to key objectives of the research and data presentation. Figure 1 over the page gives a summary of the policy consideration towards data access, control, storage and curation.

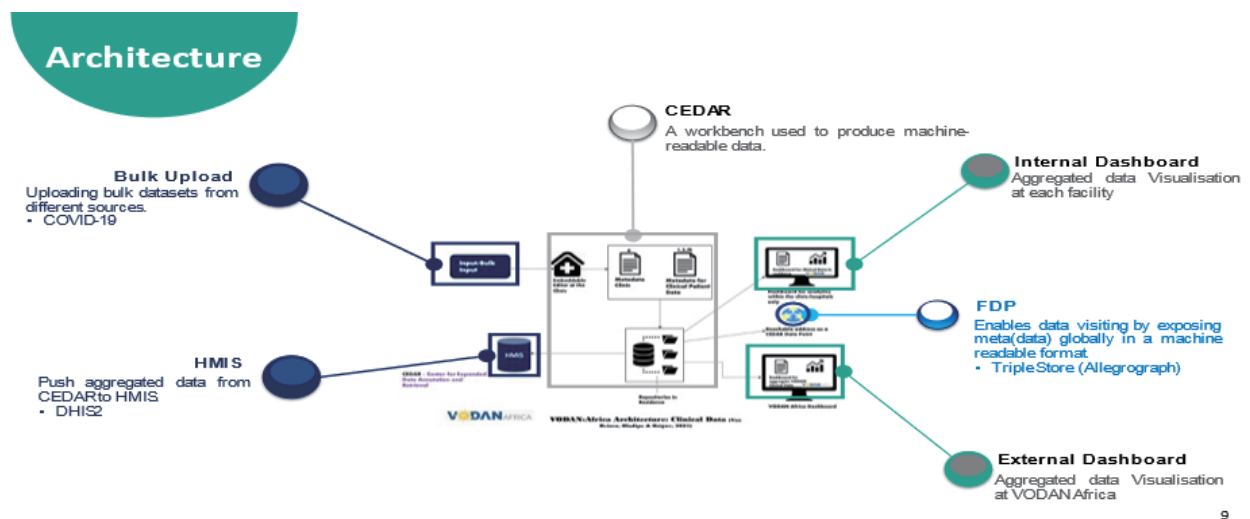


Figure 1. A diagram showing a synopsis of an elaborated architecture for data ownership and retention in places where data is produced.

A summary of the policy consideration towards data access, control, storage and curation:

1. Ownership of data in the place where it is produced.
2. Localization of data, to make sure provenance is retained
3. Regulatory compliance based on specific requirements in the location where data is produced and re-positied.

It was concluded from the study that a health data architecture should promote data ownership, localization, and regulation compliance (OLR), which is described as FAIR-OLR [15] within the FAIR-specifications.

A key outcome of the VODAN-Africa was the development of a software infrastructure that enabled data to be curated according to the FAIR-OLR principles. The localized software was developed on the basis of CEDAR Metadata Centre (<https://metadatacenter.org/>) and the tests of it demonstrated that it allowed algorithms to visit the data held in health facilities in different places across borders in federated format, based on prior permissions obtained [27].

On the basis of the outcomes obtained on the development of data held in location, the team developed the concept of the Africa Health Data Space as a transnational space which connects data curated as FAIR quality ethical Africa health data for pandemic preparedness and quality of care for vulnerable populations. Inspired by the aspiration of the development of a European Health Data Space which aims to regulate the transmission and sharing of health data across the EU for both private individuals and researchers or policy makers, the African Health Data Space will have a similar role for Africa. In order to unleash the full potential of health data for the individuals and society in Africa a Health Data Space should be developed in parallel to the European Health Data Space being developed at this stage. It aims to foster health research, reduce health care costs and produce quality data following FAIR principles.

So far nine countries have joined and the health facilities participating in VODAN-Africa. The African Health Data Space will lay the foundation to ensure that the services are contributing to better informed health care. Health facilities will benefit from the use of data in the health facilities and the data analytics across the facilities. The objective is to create a better representation of patients in remote and vulnerable settings. It stimulates a data space which is set up with inclusiveness (including data from facilities in remote and vulnerable settings).

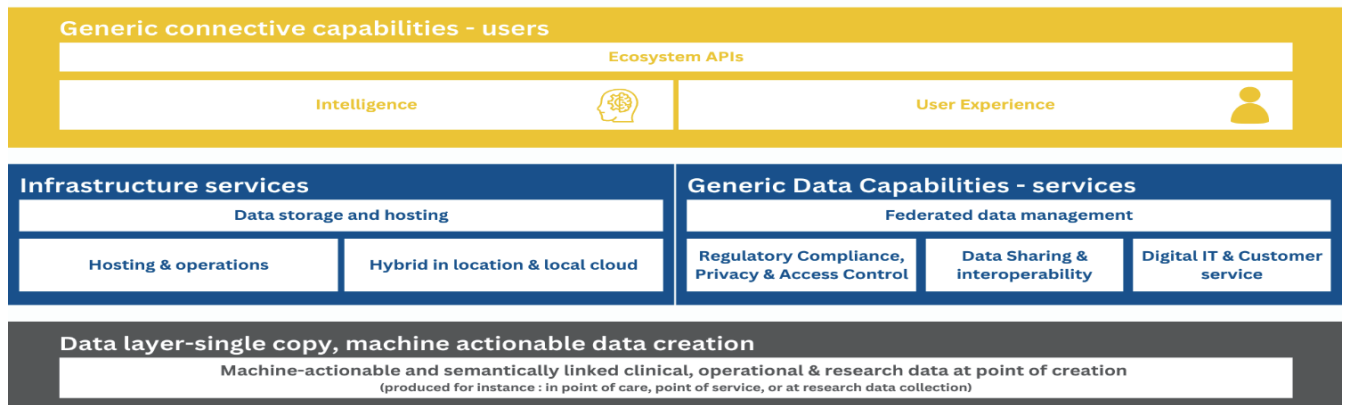


Figure 2. Architecture of a Federated Health Data Space as a proposed landscape (Van Reisen, Amare, Gebremeskel, Plug & Stocker, 2023).

The innovation of digital health solutions is expected to better serve to improve health outcomes. Hence, it should help provide solutions for the following problems:

1. Lack of data-ownership on the African continent and migration of data away from care providers and data subjects;
2. Lack of data-use and reuse, including for quality of care at the level of health facility;
3. Inadequacy, lack of representation and poverty-based bias of data from Africa in global health analytics, impeding pandemic preparedness;
4. Poverty-based bias in health data from African which lead to further exclusion of people living in poverty, which are poorly represented in the development of digital AI based health tooling innovation;
5. Lack of interoperability of data for the use of health care providers, due to vertical digital upstream solutions that do not integrate at the level of point of care and preventative medicine;

An African Health Data Space is based on health data curated as Findable, Accessible (under well-defined VODAN-Africa conditions), Interoperable and Re-usable (FAIR) that is machine-actionable, and creates a space for strong high quality and ethical data for analytics, with the following characteristics that are better adapted to patients in these settings. This will ensure that:

1. Data can reside in the health facility where the data is produced (just as the patient records do), or held under control of the facility;
2. Data is useable for analytics within the health facility and can contribute to quality health services at point of care;
3. Data can be visited for de-identified computational analytics across health facilities and across borders through algorithmic data visiting;
4. The creation of an inclusive data pipeline across larger and smaller areas and facilities in connected and remote areas will increase representativeness of the data, and remove poverty-based bias and assist in pandemic preparedness;
5. The creation of interoperable health data will increase the relevance of digital health innovations and assist in horizontal integration of these innovations.

There are various ways proposed to realize this space through digital innovations that are coming up and this will be a source for new generation of economic opportunity. VODAN-Africa concluded that it should support this as a conduit for the channeling of research grants to local universities in Africa and help build the expertise necessary to create and build relevant ethical and locally owned data infrastructures.

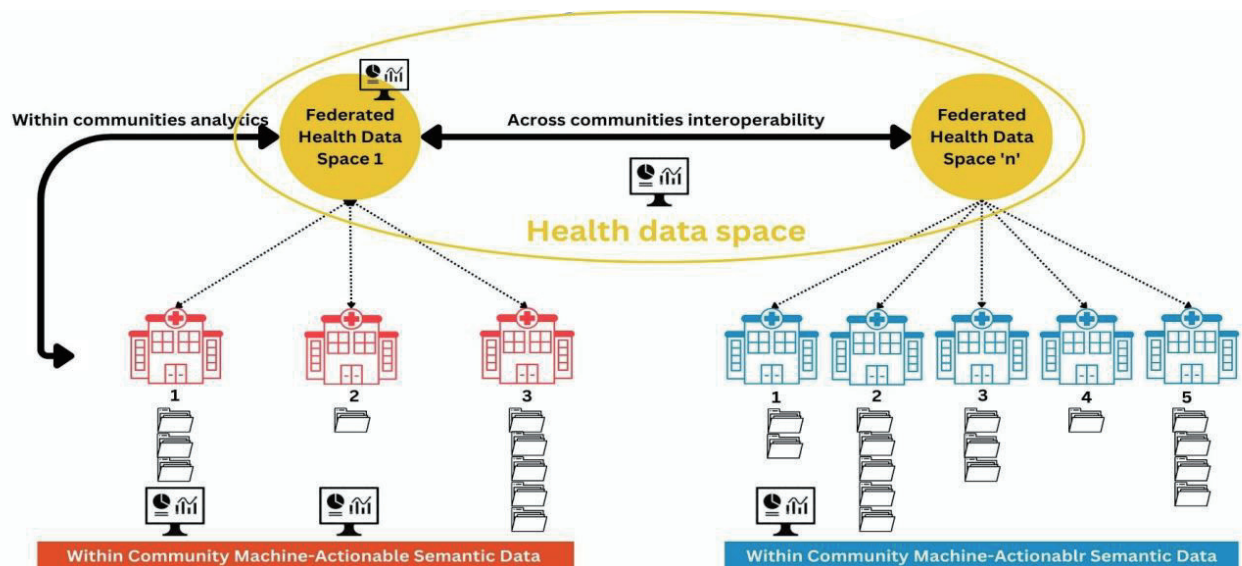


Figure 3. FAIR-OLR Federated analysis and learning.

The mission of VODAN-Africa is to create open-source innovation by African engineers and health workers to enhance health data interoperability problems in Africa. The VODAN-Africa works closely with researchers from Europe, Asia and the Americas and is linked in to the research carried out at Leiden University on the use of FAIR-Data for improved health outcomes. The outcomes from the VODAN-Africa community of practices are leading to the wider perspective in creating Africa Health Data space.

The VODAN-Africa approach showed curation of comprehensive data which were supportive of Kenya government policy on interpretation of SDG3 within the wider health sector. A case in point is the need to identify a proper health infrastructure tool which would store data within all hospitals to strengthen the Kenyan capacity for research, vaccine and medicine development and patient treatment.

4. Discussion

The establishment of the Virus Outbreak Data Network (VODAN)-Africa, was triggered by past experiences on data loss, following the Ebola crisis in West Africa in 2014. The international Community resolved to assist with a great investment in diverse digital data. The moment the crisis was solved, the digital capacities migrated, leaving behind a weakened health infrastructure. The diverse health digital data quickly moved with the experts to Europe and USA. The accessibility of data was not there. When the global fraternity started experiencing the Covid-19 pandemic, there was immediate concern not to repeat the mistake of 2014. There was immediate demand for global health data when the Covid-19 pandemic broke out. The realization was that data from Africa was not reflective of many remote or accessible areas nor included people who did not access health testing centers.

Another challenge emerged, as observed by the VODAN-Africa research teams. The work undertaken in health facilities in Africa on digitization of patient data, where this data is highly structured, rich and digitized, is normally exported for analysis by Ministries of Health and then sent to Europe and USA for further use. Once the data leaves health facilities it could not be traced nor accessed. The data remained mostly aggregated, with no allowance for more complex interoperability and reuse.

The Kenya Health Data Act defines "health data" as data related to the state of physical or mental health of the data subject and includes records regarding the past, present or future state of the health, data collected in the course of registration for, or provision of health services, or data which associates the data subject to the provision of specific health services, as earlier discussed [24]. We shall use this definition of health data for this discussion.

The Constitution of Kenya states the right to health is a fundamental human right and provides that every person has the right to the highest attainable standard of health, including the right to health care services [24]. This is also reflective of the Sustainable Development Goal 3 which aims to ensure healthy lives and well-being for across all stages of life [24]. Kenya is one of the countries that has a policy around preparing national Health

data standards and interoperability guides. The Kenya Government created a Kenya ICT Authority in 2016, which covers health data too. The Government of Kenya has identified ICT as a catalyst to attaining efficiency in healthcare service delivery and picks out ICT as a major catalyst for achieving efficiency in the multiple facets of better health service delivery.

Most health facilities in Kenya used District Health Information System (DHIS) 2. This system had many limitations. Other than the DHIS2 serving as a platform for Health Management Information System Software for routine aggregated health data from the lower health facility up to the Ministry of Health, DHIS2 could not capture all business processes starting from registration up to medication dispensation. The limitation experienced was in collecting patient level data.

4.1. A new path opening for policy on health data in Kenya

While most countries are preparing for national health data standards and interoperability guide lines like Kenya's ICT Authority, there is limited practices on the ground. At the completion of the VODAN-Africa study and implementation, over 88 health facilities used as part of the study and with 77 able to produce data in residence. The Integration of the health data life cycle called for enhancing quality of care in health facilities by enabling reuse and interoperability of data by health workers while also assisting with analysis of health data across health facilities for various purposes and use cases based on different algorithmic-pathways.

Therefore, to realize this there was the development of a technical infrastructure to enhance Findability, Accessibility, interoperability and Reusability, usually referred to as data FAIR of digital resources such as patient data, for both human and machines, called FAIRification, which allows learning from data without data leaving the residence or placed of provenance based on Ownership, Localization and Regulatory compliance. The VODAN-Africa research technical teams were able to create an architecture which could be used for scientifically obtained data and make sure data sets are interoperable, by using the same VODAN-A-Portal (same bio portal resource). By implication, VODAN-Africa has made a strong case that with proper investment and support to research centers in Africa, it is possible to collect, store and access health data curated by respective health ministries and scientific data.

5. Conclusions

In summary, the discussion of the findings by the VODAN-Africa research team has given a rich argument why data needs to remain in residence of its production. The VODAN-Africa research team concluded that the establishment of an African Health Data Space is the next step that should be realized to improve the health infrastructure in Africa. The expansion of expertise will bring in data stewards, unemployed youth and support the government concern for teaching in vocational education training in data infrastructure development and knowledge transfer.

The paper has made the analysis that the continent of Africa will no longer be a spectator excluded from representation and will be strongly represented at the global health level. Without data being curated at its point of production most developing countries, would continue in their poverty perpetuity. Africa is now ready to lead in the emerging tech community. It means that growth in data intelligence should open opportunities for new innovations that can help developing countries dig into this untapped healthcare economic empowerment of its unemployed youth.

The paper has made fundamental points by challenging existing paradigms on value creation for the health sector. The findings by VODAN-Africa show that developing countries have the potential to have a competitive advantage in digital health data production and sharing with the international community. By controlling their own digital health data, the shift in the paradigm of economic development in countries of Africa and Asia has just started, where now, the value of health data can be invested in health services and exchanged for money as a resource owned by the health facilities who produce and curate the data.

Author Contributions:

Dr Reginald Nalugala works at Tangaza University in Nairobi, where he specializes in social transformation across Africa. The key interests touch on digital health data for efficient accessibility by the patients as co-owners of the data and safe storage of that data within place of production. He also addresses data coming from the continent around refugees, migrants, conflicts,

underdevelopment, role of Faith based organizations and the development of Africa. The extractive industry and exploitation of the continent make it important to collect sound data for social transformation.

Professor Dr. Mirjam van Reisen hold the chair FAIR Data Science at the Leiden University Medical Centre. She previously held the chair Computing for Society at the Leiden Institute for Advanced Computer Science. Mirjam van Reisen is also Prof International Relations, Innovation and Care at Tilburg University Faculty of Humanities and Digital Sciences, Department of Culture Studies.

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Data Availability Statement: Sources can be found at Vodan Africa website at <https://www.vodan-totafrica.info/>

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Prognostic inspection for proactive maintenance

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Abstract: Preventive medicine aims to promote health by investigating and researching the distribution of health and health-related risk factors. The desired life time of the small modular reactor (SMR) being developed as a new energy source is more than 100 years. Improving the problems of key components such as stress corrosion cracking (SCC) and corrosion fatigue of the SMR structures and erosion and fretting fatigue of turbine blades improves the service life of the SMR. Also, in bearings, it improves wear and rolling/sliding contact fatigue. It is necessary to develop a proactive maintenance program prior to design to ensure that the SMR and bearing systems can be shut down after operation within their designed lifespan. Similar to preventive medicine, proactive maintenance programs applied to the SMR and bearing systems must undergo advance inspection. This ensures that the characteristics of key components do not deteriorate or exceed predetermined standards. Additionally, it is necessary to develop and standardize technology that restores the characteristics of key components to their designed performance state. In this paper, we introduce the concept of prognostic inspection and proactive maintenance (PIPM) system with ultrasonic nanocrystal surface modification (UNSM) and suggest a method of applying PIPM to SMR and bearing systems.

Keywords: Prognostics and Health Management (PHM), Prognostic Inspection and Proactive Maintenance (PIPM), Ultrasonic Nanocrystal Surface Modification (UNSM), Small Modular Reactor (SMR), Bearing

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1. Introduction

Devices and systems used in various industrial fields are damaged for various reasons even when they are optimally designed. Preventive maintenance is the process of periodically replacing parts to prevent this. However, it requires high costs every year and is not excellent at preventing accidents. Recently, as the use of big data and machine learning has increased, Prognostics and Health Management (PHM), a technology for diagnosing and prognosis fault, is being researched and applied [1-4]. In the medical field, biomarkers have been used for a long time to personalize medicine or health management and analyze the safety of medicines. These biomarkers are produced either by organs affected by the disease or by the body in response to various diseases. They are utilized to monitor the progression of a disease [5, 6]. The population health management requires advanced information technology systems and tools that can collect, store, process, and analyze large amounts of health data, and biomarkers are an important element of the population health management [7]. PHM in the industrial field also identifies and monitors factors that affect the progression of damage, such as biomarkers. After analyzing the values of these factors for the current state using big data-based data, it is decided whether or not to carry out maintenance. The application of big data-based PHM is increasing in bearings, gears, and shafts in the mechanical field, based on a wide range of failure cases [8]. If the Stress Corrosion Cracking (SCC) and corrosion-fatigue problems of the Small Modular Reactor (SMR), which is being developed as a new energy source, are improved

through the PHM, the service life of the SMR will increase. SCC and fatigue problems are improved by increasing compressive residual stress rather than reducing surface roughness, and this can be achieved through surface stress improvement technology [9, 10]. In this paper, we introduce the concept of Prognostic Inspection and Proactive Maintenance (PIPM) system with Ultrasonic Nanocrystal Surface Modification (UNSM) and suggest a method of applying PIPM to SMR and bearing systems.

2. Prognostic Inspection and Proactive Maintenance (PIPM) Systems

2.1. Different types of maintenance

Reactive Maintenance (RM) only repairs or replaces machine parts when an error occurs and they can no longer function. The advantage of this method is that the cost associated with maintenance personnel and maintaining machine operation is low. However, since the machine or parts are used until they break down, the probability of serious failure increases, and the repair cost also increases [11]. Scheduled Maintenance (SM) is a maintenance method performed at regular time intervals. The goal is to perform maintenance activities even while the machine is operating under normal conditions to minimize the possibility of failure and prevent costly unplanned downtime. On the other hand, the SM method requires performing some costly maintenance interventions even when the equipment is still functioning properly [4]. Condition Based Maintenance (CBM) and PHM both focus on maintaining system reliability and reducing system downtime. CBM is a maintenance strategy that uses sensors and data analytics to monitor the real-time performance of assets or equipment. CBM uses real-time data to determine maintenance requirements and perform maintenance, making it a more efficient maintenance method than RM and SM [12]. PHM, on the other hand, is a technology that enables CBM and has the ability to predict the Remaining Useful Life (RUL) of a system while it is in operation. PHM is a new maintenance approach that only handles repairs or replacements for actual damage to components [13]. The most common applications of PHM in the mechanical field are bearings, gears, and shafts. As shown in Table 1, PHM is implemented through algorithm analysis by collecting failure modes, characteristics, and common features [8, 14].

Table 1. Summary of PHM of mechanical components

Component	Failure mode	Characteristic	Common feature	Common algorithms used
Bearing	Outer-race, inner-race, roller, cage failures	Raw data does not contain insightful information; low amplitude; high noise	Vibration characteristic frequency, time domain statistical characteristics, metallic debris shape, size, quantity, sharp pulses and rate of development of stress-waves propagation	Fourier Transform (FT), Short Time Frequency Transform (STFT), Wavelet Transform (WT), etc.
Gear	Manufacturing error, tooth missing, tooth pitting/spall, gear crack, gear fatigue/wear	High noise; high dynamic; signal modulated with other factors (bearing, shaft, transmission path effect); gear specs need to be known	Time domain statistical features, vibration signature frequencies, oil debris quantity and chemical analysis	Fourier Transform (FT), Short Time Frequency Transform (STFT), Wavelet Transform (WT), etc.
Shaft	Unbalance, bend, crack, misalignment, rub	Vibration signal is relatively clean and harmonic frequency components of rotating speed can indicate the defects	Vibration characteristic frequency, time domain statistical characteristics, system modal characteristics	Fourier Transform (FT), Wavelet Transform (WT), etc.

2.2. PIPM systems

The Prognostic Inspection and Proactive Maintenance (PIPM) system is an integration of the prognostic inspection methodology and the proactive maintenance methodology. The proactive maintenance methodology entails

the maintenance aspects which should be incorporated at the design stage of a physical asset, whereby all anticipated probable prospective failures are identified and removed, based on the focused historical performance of the asset, with the purpose to prescribe suitable maintenance actions at the right intervals and component parts [15]. So the PIPM system should be developed also from the design phase, especially for the regulator’s safety evaluation in Design Certification Application (DCA) [16].

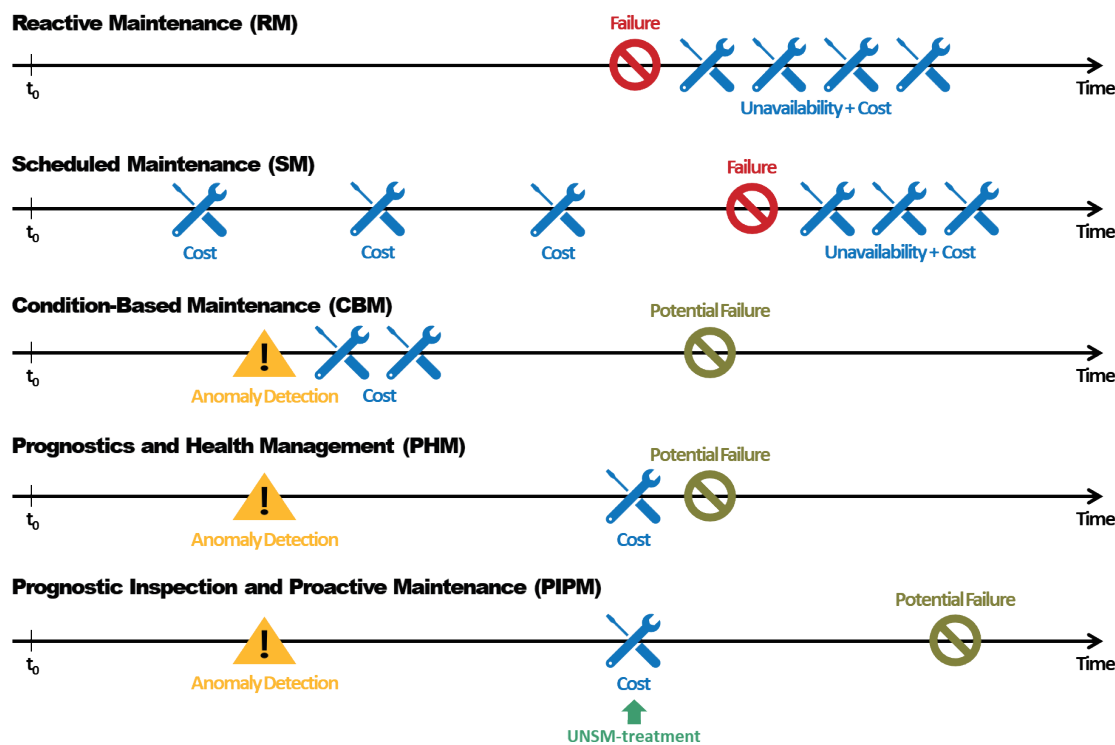


Figure 1. Scheme of the behavior of the different type maintenance approaches [4].

The concept of prognostic inspection is similar to that of CBM, which is an advanced form of preventive maintenance [1, 2]. However, an important difference lies in the measurement focus. Instead of measuring crack growth conditions, the PIPM system focuses on crack initiation conditions. To implement this, a method for measuring the condition of components at the onset of failure mode, for example, the condition of crack initiation, needs to be developed. In CBM and PHM, crack growth conditions are already measured using analysis algorithms through changes in vibration, noise, etc. Measuring crack initiation conditions is difficult, but if possible, the potential failure interval can be significantly increased. The purpose of the PIPM system is to determine crack initiation conditions and delay crack initiation with a proactive maintenance method using UNSM technology, thereby increasing the potential failure interval and service life of the product. Consideration should be given to new sensor technologies integrated with Artificial Intelligence (AI), which could be available in the future, for the development of prognostic inspection methodology. Furthermore, the remaining service life until the beginning of crack initiation should be estimated based on the results of these measurements by the analyzing function.

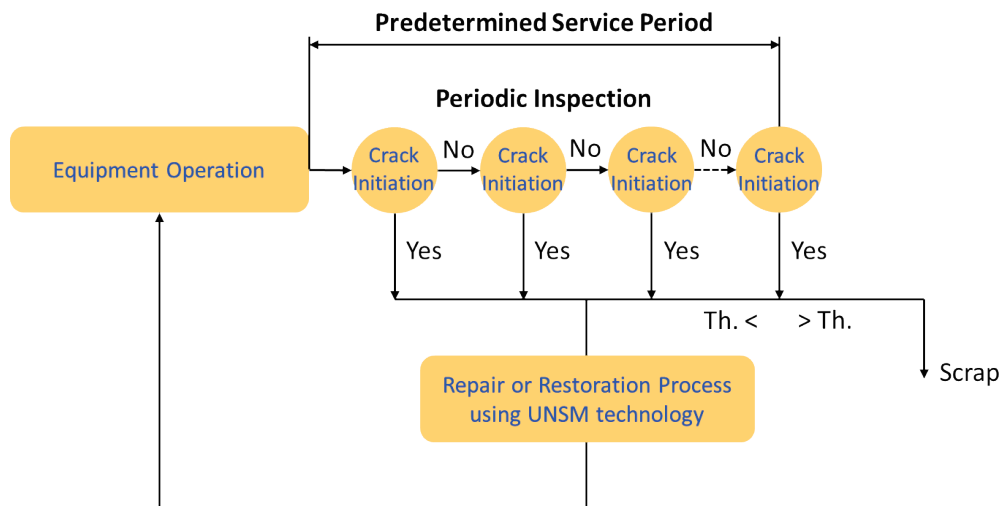


Figure 2. Flow of the PIPM system.

Proactive Maintenance involves a variety of approaches and technologies aimed at significantly reducing reactive maintenance in practice. The defining characteristic of proactive maintenance is the exploration of failure origins. Repair or restoration processes delay potential failures by eliminating the cause of the failure. The technology to be used in this process is UNSM technology that can improve surface stress. The UNSM is a surface modification/treatment technology that causes surface severe plastic deformation at the surface region to generate a gradient nanostructured surface layer along with a modified surface [17]. This technology has already been proven to improve the service life of industrial parts such as knives and bearings, and with the approval of ASME Code & Case N931, it is highly likely to be applied to nuclear power plant components. Proactive maintenance is highlighted as an innovative strategic maintenance approach that aims to significantly improve the reliability and availability of components throughout their service life [18]. A proactive maintenance methodology should be developed that can be applied to components during refueling outages and could restore the fatigue strength of components to the condition of new components. Consideration should also be given to new and advanced technologies that could be available in the future as potential candidates for the proactive maintenance methodology.

Table 2. Typical examples of the PIPM system

Prognostic Inspection and Proactive Maintenance (PIPM) system		
Component	Prognostic Inspection Methodology	Proactive Maintenance Methodology
Bearing	<ul style="list-style-type: none"> Sensing and Monitoring of Vibration, oil debris, Acoustic emission, Energy, Temperature, etc.: usually continuous monitoring [14] Measuring and Monitoring of Surface roughness, Micro-cracks, Hardness, Residual stress, Grain size, etc.: usually inspecting during the refueling outage [19] Analyzing the remaining service life till the beginning of crack initiation based on the measuring results Deciding whether sustaining till next or next nth refueling outage or applying the proactive maintenance 	<ul style="list-style-type: none"> Exchanging with the new bearings or the remanufactured bearings whose service life is equivalent to new bearing during the refueling outage [22] Restoring the bearings using UNSM (Ultrasonic Nanocrystal Surface Modification) technology during the refueling outage
SMR Component	<ul style="list-style-type: none"> Visual inspection of discontinuities and imperfections on the CISCC (Chloride Induced Stress Corrosion Cracking) susceptible surfaces; usually measuring during the in-service inspection period [20] Measuring and Monitoring of Surface roughness, Residual stress, etc.: usually measuring during the in-service inspection period [21] Analyzing the remaining service life till the beginning of crack initiation based on the measuring results Deciding whether sustaining till next or next nth in-service periods or applying the proactive maintenance 	<ul style="list-style-type: none"> Restoring the necessary surface condition by cold splay technology or advanced surface stress improvement technology such as UNSM, Laser Peening, etc. in ASME Code & Case N931 [23]

3. Conclusions

The PIPM system is a concept that integrates prognostic inspection methodology and proactive maintenance methodology. The key point of the PIPM system is to determine crack initiation conditions and combine repair or restoration processes to improve the service life of the product. This is a model pursued in the circular economy system and an eco-friendly system model to be used in the fields of steel, nuclear power plant and transportation. However, the development of prognostic inspection technology that determines crack initiation conditions must be modeled through the development will and necessity of the main organization. In addition, the PIPM system should be advanced by securing reliable verification of fatigue and life restoration of UNSM technology, the most representative surface stress improvement technology. It is expected that the advanced PIPM system will be applied as a technology required for SMR and bearing system preventive maintenance in the future.

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Article

Impact of Environmental Exposure to Air Pollutants at Workplace on Respiratory Health of Dust-exposed Congolese Workers

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Abstract: WHO considers ambient air pollution as the greatest environmental health risk. The “United Nations Environment Program Action Notes 2021” reported that 99% of world population live in places with atmospheric air pollutants levels above WHO’s exposure limits. This mini-review included three studies from the “Congo Occupational/Environmental Health Research Project 2016-2020”, focusing on the respiratory health of dust-exposed workers. They are case-control studies conducted by our Congo-Japan collaborative research team in stone quarry sites and construction sites in Katanga region, which is part of the African cooper-belt region. In total, 1,512 workers participated in those studies; the first one included 512 workers (256 female stone quarry workers, 256 unexposed female workers), the second included 570 workers (282 male cement conveyors at construction sites, 288 unexposed male stone quarry workers), whereas the third study included 441 workers (199 dust-exposed male artisanal coltan miners, 242 unexposed male workers). Control groups comprised local administrative office workers and market tax collectors. Participants answered a respiratory health questionnaire, underwent physical examination and lung function testing. Air quality was assessed by means of an air quality monitor. Results showed a lack of personal protective equipment use in both female stone quarry workers and male cement conveyors. Our first study conducted in stone quarry sites which included female workers, higher PM_{2.5} ($205 \pm 13.2 \mu\text{g}/\text{m}^3$ vs. $31.3 \pm 10.3 \mu\text{g}/\text{m}^3$; $p < 0.001$) was observed, as compared with controls. Similarly, the second study which included male cement conveyors also showed higher PM_{2.5} ($197.0 \pm 0.0 \mu\text{g}/\text{m}^3$ vs. $29.0 \pm 0.1 \mu\text{g}/\text{m}^3$; $p < 0.001$), as compared with unexposed controls. Furthermore, third study which included male coltan miners showed extremely elevated PM_{2.5} ($215.0 \pm 11.3 \mu\text{g}/\text{m}^3$ vs. $33.0 \pm 4.2 \mu\text{g}/\text{m}^3$; $p < 0.001$) compared to control workplaces. Respiratory complaints were very common in all exposed workers’ groups, with significantly reduced lung capacity in female stone quarry workers (mean PEFr: 344.8 ± 2.26 vs. 405 ± 67.7 L/s; $p < 0.001$), hand-operated cement conveyors (445.1 ± 89.0 vs. 482.3 ± 63.2 ; $p < 0.001$) and artisanal coltan miners in the third study (347.9 ± 66.9 vs. 493.674 ; $p < 0.001$). Dust-exposed Congolese workers are exposed to high air pollutants levels, which contribute to increased frequency of respiratory complaints and impaired lung function.

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Keywords: Air pollutant, Construction worker, Particulate matter, Quarry worker, Respiratory health.

1. Introduction

According to the World Health Organization (WHO), environmental air pollution is one of the major health threats.

WHO considers ambient air pollution as the greatest environmental health threat. A report from the “United Nations Environment Program (UNEP) Action Notes 2021” stipulates that 99% of world population live in places with atmospheric air pollutants levels above WHO’s exposure limits [1, 2]. Exposure to high particulate matter (PM) levels are associated with lung function impairment. A recent Indian study conducted among cement dust-exposed workers showed markedly decreased lung function capacity in exposed workers compared to controls, and a positive association between cement dust exposure and low peak expiratory flow rate [3].

Petrochemical industry, open and underground mining industries, cement factory and construction industries are among the major contributors to ambient air pollution (AAP) in countries of the sub-Saharan Africa region. A few studies conducted in Nigeria showed that quarry workers with a history of chronic dust-exposure had a greater risk of developing respiratory symptoms [4, 5]. Furthermore, studies conducted among dust-exposed workers from gold, diamond and platinum mining sectors showed high proportions of miners who developed silicosis, a chronic lung disease caused by silica dust [6].

In the Democratic Republic of the Congo (DRC), studies that explored the respiratory health of workers exposed to mineral dust are scarce. The present report is a review that includes studies that assessed the air quality in stone quarry, construction and artisanal coltan mining sites, and evaluated the respiratory health of exposed workers in Lomami and Haut-Katanga, formerly Katanga region.

2. Materials and Methods

2.1. Study design, sites and participants

This was a mini-review that included three studies, focusing on the air quality monitoring in working environment and the respiratory health of dust-exposed workers in DRC. They are case-control studies from the “Congo Occupational/Environmental Health Research Project 2016-2020” [7–9], focusing on the respiratory health of dust-exposed workers. These studies were conducted by our Congo-Japan collaborative research team in stone quarry sites and construction sites in Katanga area, which is part of the African cooper-belt region. In total, 1,512 workers participated in those studies; the first one included 512 workers (256 female stone quarry workers, 256 unexposed female workers), the second included 570 workers (282 male cement conveyors at construction sites, 288 unexposed male hand-operated cement conveyors), whereas the third study included 441 workers (199 dust-exposed male artisanal coltan miners, 242 unexposed male workers). Control groups comprised local administrative office workers and market tax collectors. Katanga region is located in southern area of DRC; it is part of the African copper-belt, which also includes a part of Zambia (Fig. 1) [10].

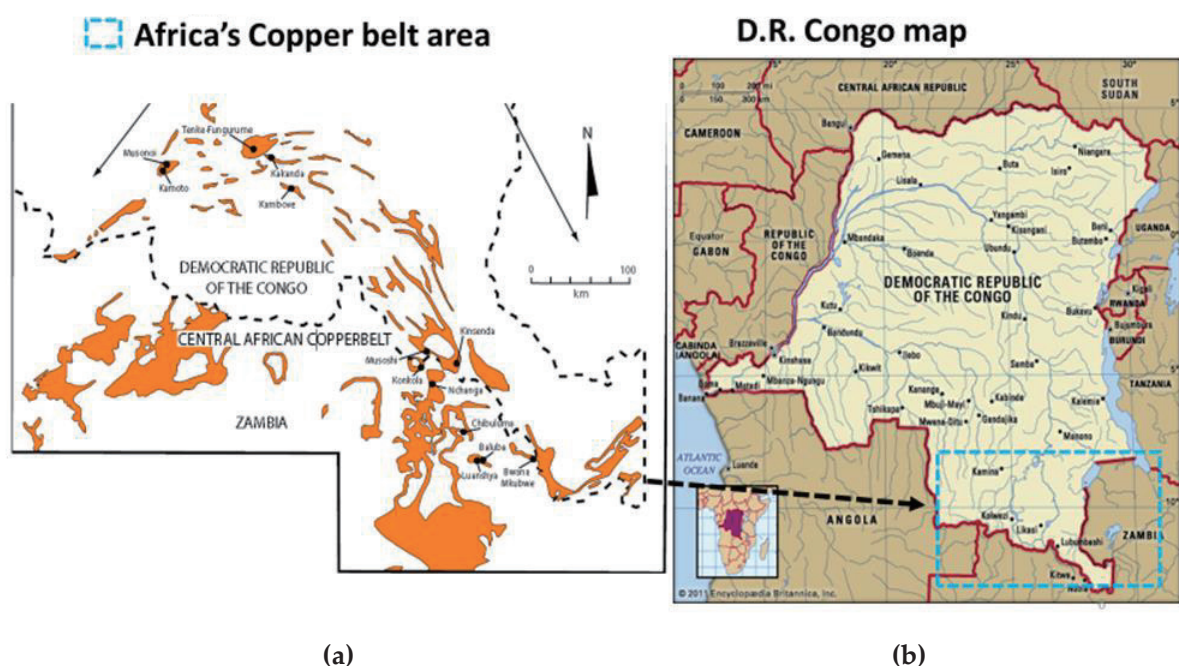


Figure 1. (a) Central African copper belt area; (b) map of the Democratic Republic of the Congo.

2.2. Inclusion criteria

Inclusion criteria were as follows: voluntary participation, having at least one year of work experience, absence of contraindication for lung function test, provide informed consent, be present at workplace on the day of examination, not participating in a similar study.

2.3. Survey questionnaire, lung function testing and air quality monitoring

Participants answered a structured respiratory health questionnaire, which comprised questions related to anthropometric, sociodemographic and occupational characteristics, and respiratory health symptoms. In addition to a general medical check-up (including chest auscultation), lung function test was performed with the use of a peak flow meter. Peak expiratory flow rate (PEFR) was the parameter considered in this report. Air quality assessment was carried out three times at each quarry site, with a 30-minute interval, using BRAMC air quality monitor (BR-AIR-329). The concentrations of atmospheric particulate matter (PM_{2.5}) and volatile organic compounds (VOC) were the air pollutants measured and considered in this report. In each of the research protocols of the three studies included in this report, hygrometric or meteorological measurements were not planned.

2.4. Ethical consideration and statistical analysis

To participate in this study, each subject provided a written informed consent; participation was voluntary. Ethical approval of the DRC OSH research project was obtained from the ethics committee of the School of Public Health, University of Lubumbashi, DRC (approval number: UNILU/CEM/075/2015).

Group comparisons were performed using Student's t-test for continuous variables, whereas Fisher's exact test was used for categorical variables. Multivariate logistic regression analysis was performed, with adjustment for sociodemographic characteristics such as age, education level and smoking status, in order to determine factors associated with respiratory manifestations. Stata software v.15 was used for data analysis and the level of significance was set at p-value less than 0.05 (double-sided).

3. Results

3.1. Air quality in the working environments

It was observed that personal protective equipment (PPE) was not used by stone quarry workers and cement conveyors. Figure 2 presents results of environmental monitoring of atmospheric levels of PM_{2.5} and VOC. It was observed that stone quarry (205.0 ± 13.2 vs. 31.3 ± 10.3 $\mu\text{g}/\text{m}^3$), construction (215.0 ± 11.3 vs. 33 ± 4.2 $\mu\text{g}/\text{m}^3$) and artisanal coltan mining sites (197 ± 0.0 vs. 29.0 ± 0.1 $\mu\text{g}/\text{m}^3$) had markedly higher PM_{2.5} levels as compared with corresponding control sites ($p < 0.001$). Regarding atmospheric VOC, though relatively high levels were found in stone quarry and construction sites, as well as artisanal coltan mining sites as compared with control sites, no statistically significant difference was observed.

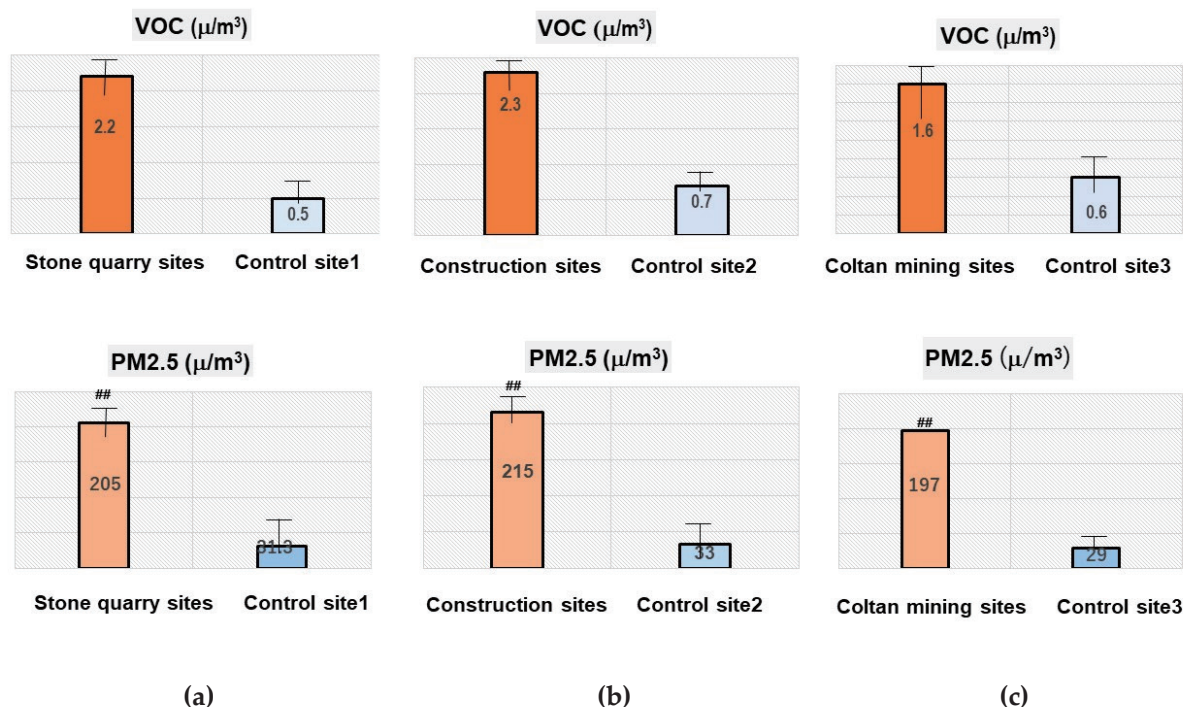


Figure 2. Mean values of workplace PM_{2.5} and volatile organic compounds (VOC) levels in (a) stone quarry sties; (b) construction sites; (c) artisanal coltan mining sites in Katanga region, Democratic Republic of the Congo.

3.2. Characteristics of the study participants and air quality in the working environment

As shown in Table 1, a large proportion of workers from the exposed groups were current smokers; no statistically significant difference was observed when comparing mean age between exposed workers and controls. Respiratory manifestations were very common in all exposed workers’ groups. Asthma was the respiratory disorder that was significantly more prevalent in coltan miners, 32.2% (vs. 2.1% in controls; $p < 0.0001$), chronic bronchitis was more prevalent in female stone quarry workers, 17.6% (vs. 1.1% in controls; $p < 0.001$), whereas rhinitis was more prevalent in female stone quarry workers (57.8% vs. 13.4% in controls; $p < 0.0001$) and coltan miners (70.3% vs. 17.7% in controls; $p < 0.0001$).

Table 1. Sociodemographics, frequency of respiratory manifestations , workplace pollutants and lung function status among dust-exposed Congolese workers (stone quarry workers, artisanal coltan miners) (N= 1,523)

Characteristics of participants (N=1,523)	Female Stone Quarry Workers (n1= 512)		Hand-operated Cement Conveyors (n2= 570)		Male Coltan Miners (n3= 441)	
	Controls (n=256)	Exposed (n=256)	Controls (n=288)	Exposed (n=282)	Controls (n=242)	Exposed (n=199)
1. Sociodemographics						
Age (mean; SD)	44.1 (9.4)	43.1 (0.8)	33.8 (8.6)	32.5 (8.4)	33.8 (9.2)	32.8 (8.4)
Gender						
F (n; %)	256 (100)	256 (100)	0 (0)	0 (0)	0 (0)	0 (0)
M (n; %)	0 (0)	0 (0)	288 (100)	282 (100)	441 (100)	441 (100)
Working years [mean (SD)]	8.3 (7.1)	2.92 (4.5)	6.4 (6.7)	4.7 (4.4)	5.66 (6.7)	5.7 (3.5)
Education level (n; %):						
-Highschool not completed	197 (76.9)	245 (95.7)	252 (87.5)	270 (95.7)	155 (64.0)	140 (70.4)
-Highschool or higher	59 (23.1)	11 (4.3)	36 (12.5)	12 (4.3)	87 (59.6)	59 (29.6)
Current smokers (n; %)	9 (3.5)	2 (0.8)	16 (5.6)	169 (59.9)	22 (4.1)	5 (7.8)

2. Respir. manifestations						
Rhinitis (n; %)	25 (13.4)	148(57.8)##	53 (18.4)	226(80.1)##	43 (17.7)	140(70.3)##
Chronic bronchitis (n; %)	2 (1.1)	45 (17.6) #	2 (0.7)	11 (3.9)	-	-
Asthma (n; %)	3 (1.6)	6 (2.3)	3 (1)	8 (2.8)	5 (2.1)	64(32.2)##
Morning cough (n; %)	6 (2.5)	95 (47.7) ##	43 (14.9)	144 (51.1)#	-	-
Morning sputum (n; %)	3 (1.6)	105 (41) ##	32 (11.1)	124 (44)#	8 (3.3)	99 (49.7) ##
Dyspnea at rest (n; %)	2 (1.1)	80 (31.3) ##	37 (12.8)	92 (32.6)	6 (2.5)	55 (27.6) ##
Dyspnea after effort (n; %)	1 (0.5)	99 (38.7) ##	-	-	6 (2.5)	60 (30.1) ##
Wheezing at rest (n; %)	17 (9.1)	69 (2.7)	14 (8)	51 (18.1)	35 (14.5)	85 (42.7) #
Wheezing after effort (n; %)	17 (9.1)	57 (22.3)	-	-	24 (9.9)	67 (33.7) #
3. Lung Function Test						
Peak Expiratory Flow Rate (PEFR; L/s) (mean, SD)	405 (67.7)	344.8 (2.3)#	482.3(63.2)	445.1 (89)#	493.2(67.4)	347.9(66.9)#

Notes: # denotes $p < 0.01$; ## denotes $p < 0.001$

Regarding respiratory complaints, morning cough (47.7% in female quarry workers vs. 2.5% in controls; 51.1% in cement conveyors vs. 14.9% in controls), morning sputum (41% in stone quarry workers vs. 1.6% in controls; 44% in cement conveyors vs. 11.1% in controls; 49.7% in coltan miners vs. 3.3% in controls), dyspnea at rest (31.3% in stone quarry workers vs. 1.1% in controls; 27.6% in coltan miners vs. 2.5% in controls) and wheezing at rest (42.7% in coltan miners vs. 14.5% in controls) were more frequent in dust-exposed workers than controls (Table 1). Furthermore, markedly reduced lung function capacity was observed in the first study that included female stone quarry workers (mean PEFR: 344.8 ± 2.26 vs. 405 ± 67.7 L/s; $p < 0.001$), hand-operated cement conveyors in the second study (445.1 ± 89.0 vs. 482.3 ± 63.2 ; $p < 0.001$) and artisanal coltan miners in the third study (347.9 ± 66.9 vs. $493.67.4$; $p < 0.001$) (Table 1).

Table 2. Association between dust-exposure status and respiratory manifestations by logistic regression in Congolese stone quarry workers and informal coltan miners (adjusted analysis)

Respiratory manifesta-tions	Quarry work		Construction work (cement)		Coltan mining	
	aOR (SE)	95% CI	aOR (SE)	95% CI	aOR (SE)	95% CI
Morning cough	6.1 (1.9)	3.29-11.15#	3.39	2.12-5.41#	12.0 (3.7)	3.3-24.50#
Morning phlegm	2.6 (1.0)	1.24-5.52*	3.66	2.19-6.09#	4.7 (3.0)	2.45-11.62#
Wheezing after effort	3.4 (1.6)	1.28-8.78*	2.27	1.21-4.25*	0.3 (0.0)	0.09-0.78
Dyspnea at rest	3.9 (1.1)	2.22-7.02#	-	-	4.0 (2.1)	1.16-15.53*
Dyspnea after effort	5.2 (1.9)	2.46-10.84#	3.09	1.86-5.12#	4.1 (0.8)	1.21-13.73*
Rhinitis	2.1 (0.2)	1.18-3.39#	20.38	11.9-34.65#	5.5 (1.3)	4.95-6.36#
Chronic bronchitis	2.6 (0.7)	1.48-4.61#	2.06	0.33-12.08	-	-
Asthma	1.2 (0.7)	0.36-3.99	4.23	1.00-17.78*	4.9 (1.6)	2.41-17.06#

Notes: * denotes $p < 0.05$; # denotes $p < 0.01$; ## denotes $p < 0.001$; aOR is adjusted odds ratio (adjustment for age, educational level, smoking status); SE is standard error; CI is confidence interval.

Table 2 shows the results of the multivariate logistic analysis. It was observed that, compared to controls, almost all listed respiratory manifestations (except asthma) were strongly and positively associated with stone quarry work, artisanal coltan mining and construction work (for cement conveyors). For example, regarding respiratory disorders, working at stone quarry sites was associated with about 2 times higher risk for rhinitis ($p < 0.01$) and

chronic bronchitis ($p < 0.01$); workers at construction sites had 20 times higher risk for rhinitis ($p < 0.001$), whereas those from artisanal coltan mining sites had 5 times higher risk for rhinitis. Both construction work ($p < 0.05$) and artisanal coltan mining ($p < 0.01$) were associated with a 4 to 4.9 times higher risk of developing asthma-like ailment, respectively.

4. Discussion

This research project was the first that explored the respiratory health of dust-exposed stone quarry workers, cement conveyors and artisanal coltan miners in the natural resource-rich D.R. Congo. A portable air quality monitor was used to measure ambient air dust concentrations. Similar devices have been used in previous studies [11, 12]. On the hand, Peak flow meter was employed for lung function testing. It is often used in research, especially when the study site is located from a healthcare setting [13].

It was observed that environmental air concentrations of $PM_{2.5}$ work sites were significantly higher than the $25 \mu\text{g}/\text{m}^3$ exposure limit recommended by the World Health Organization. Most workers reported the lack of use of appropriate PPE against dust, particularly in regard to the protection of airways, eyes and skin (mask, goggles, gloves). Additionally, other safety measures aimed at reducing workplace dust levels were not applied at the workplaces. We also found that exposed quarry workers, cement conveyors and coltan miners had low socio-economic status and education level in general; they were exposed to high air pollutants ($PM_{2.5}$, VOC) levels, which might have contributed to increased prevalence of respiratory disorders (chronic bronchitis, rhinitis, asthma) and complaints, including morning cough, morning sputum and dyspnea.

Moreover, in the multivariate logistic regression analysis, almost all respiratory manifestations were strongly associated with stone quarry work, construction work and artisanal coltan mining. Furthermore, this research showed that exposed workers had significantly reduced lung function capacity as compared with unexposed controls. Similar findings have been previously reported in studies conducted among artisanal miners [14] and cement industry workers in other countries [15]. Those reports show not only elevated prevalence of respiratory symptoms among dust-exposed workers but also an impairment of pulmonary function due to reduced respiratory parameters. Furthermore, a Taiwanese cohort study published by Lancet Planetary Health [16] showed that chronic exposure to fine particulate matters (PM) declined lung function performance and increased the risk of respiratory disorders. This fact supports our findings in regard to stone quarry workers, cement conveyors and coltan miners in DRC.

Coltan or columbite-tantalite is a rare metal used in the manufacturing of a variety of devices, particularly in computers and mobile phone; its use in technology has been fueling the ongoing armed conflict in eastern DRC [17]. However, the health risk associated with coltan mining in Africa has been unknown. Our study was the first to investigate the respiratory health risk of coltan miners in relation to hazardous dust-exposure at the mining sites.

5. Conclusion

This mini-review highlights the poor air quality in the working environment of stone quarry, construction and artisanal coltan mining sites in the Katanga region, DRC, exposing workers to high-risk for developing respiratory disorders. The use of appropriate PPE during work by quarry workers, miners, cement conveyors and other workers in the construction industry is recommended.

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Article

Key success factors for landscape restoration and economic development using exclosures in Tigray, Northern Ethiopia

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Abstract: For decades, and in line with global initiatives, Ethiopia has made persistent efforts to reclaim its degraded lands into ecologically and socioeconomically plausible enterprises through the exclosure establishment. Tigray, a region in Ethiopia, has been a key actor in the implementation of exclosures, with immense experiences and innovations for others to learn. The region was recognized internationally and won a gold medal for its "Best Future Land Policy" in 2017. To enhance success of exclosure as a restoration strategy, deeper evaluation of critical factors is crucial. For the first time, we assessed exclosures against key success factors of land restoration through detailed discussions with 76 Key Informants and five focus groups in 19 villages of Tanqua-Abergele district of Tigray. The factors include the objective of establishment, restoration approaches, pre-exclosure land-use, levels of degradation before establishment, spatial and temporal distribution, availability of command area, time elapsed to conserve and presence of conflicts in view of understanding what attributes are in support of or missing for economic and ecological development. Analysis of restoration approach revealed that 30%, 20% and 50% of the exclosures were natural regeneration, soil and water conservation only and plantation, respectively. We found out that more than 60% of the recruited exclosures were severely degraded during establishment and calculated that 54.4% were established to comply with regional plans, 34.4% for the development of grass, water and farmlands and 10 % to buffer communal resources. While 70% have command areas, only 7% started irrigation schemes. We proved that 28, 64 and 66 sites did not meet objectives, experienced illegal grazing and tree cutting, respectively. While 75 sites are linked to another village, district or region, only five administrative units collaborated to work for a common interest and 17% experienced conflicts. We conclude that pre-establishment assessment was not properly conducted which is critical for successful exclosure development and decision on type of exclosures, their management and objective evaluation of progresses. We recommend a detailed characterization of future recruitment of degraded lands for restoration programs, considering that the land is suitable for sustainable ecological and socioeconomic development of the local population.

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Keywords: characterization, command area, economic development, exclosure, hydrological linkage, landscape restoration

1. Introduction

In Ethiopia's political and development endeavours, 1991 marks a pivotal moment for the rehabilitation of degraded lands. The large-scale establishment of exclosures as a rehabilitation strategy began after 1991 following the fall of the Derg regime in the country, although unsuccessful attempts had been made before it [1-4]. Shortly afterwards, massive exclosure establishment has been a continuous practice for the past three decades in Tigray [5, 6]. Accordingly, focused studies on success stories were tremendous out of which sustaining and scaling-up mechanisms were devised [7, 8]. For example, only 715.78 (4.12%) hectares were recruited in the Tanqua-Abergele district prior to 1991, when

the Tigray region was under the rebellion rule of the Tigray People Liberation Front (TPLF). Immediately after 1991, there was a large-scale expansion of exclosures, which reached peak in 2000-2010 and slightly decreased towards 2018 (Fig. 2 (a)) due to reasons related to exclosure sustainability [9].

Proper characterization of the exclosures is vital before scaling-up of the practice is proposed. In this study, we attempt to characterize exclosures taking defined attributes useful to be considered for successful implementation and scaling-up practice. We analyzed, for the first time, objective of establishment, restoration approaches, pre-exclosure land use, level of degradation during establishment, spatial and temporal distribution, availability of command area, time elapsed to conserve, presence of conflicts and sustainability problems in view of understanding what attributes are in support of and are missing for sustainable development (Table 1). This would be a useful step to get a broad perspective on land suitability for landscape restoration and economic development in the country and other dryland areas where countless restoration initiatives are prevailing.

Table 1. Description of Variables used to analyze the exclosure establishment and management practice in Tanqua-Abergele District of Tigray

Explanatory Variable	Description
Objective of establishment	Anticipation of the local community about what a site would achieve after its full recruitment as an exclosure as the potential of the site
Restoration approach	Whether an exclosure area was rehabilitated through (1) natural regeneration or (2) aided with construction of soil and water conservation structures or (3) assisted natural regeneration
Pre-exclosure land use	Category of land use before recruitment of an area to an exclosure
Level of degradation during establishment	Degradation status of an exclosure (no, light, moderate, severe or very severe) before the inception
Spatial and temporal distribution	Geographical location and year of recruitment of an exclosure
Availability of command area	Presence of a level land that can be used for irrigated crop, vegetable, fruit or forage development if exclosure projects enhance the availability of water
Time elapsed to conserve	Duration taken to finalize construction of soil and water conservation structures and/or vegetation enrichment of a delineated site.
Presence of conflicts	Whether the spatial distribution results in conflicts between/among villages, districts or regions

2. Materials and Methods

2.1. The Study Area

Tanqua Abergele district, the research area, is located 120 km west of the regional capital Mekelle. It shares borders with Kolla Tembien, Dequ'a Tembien and Samre districts to the North, Northeast and the South respectively, and with Amhara Regional State to the West. It is found in the lowland zones with altitude range of 932-2,394 metres above sea level. The district land administration office estimated 144,564 hectares of total land area with land-use land-cover categories of 5,466, 39740, 2,433, and 32,986 hectares being irrigation, rain-fed, pasture and exclosure areas, respectively. The remaining 63,939 hectares comprise mountains, grazing lands, settlement areas and other miscellaneous lands. From a hierarchical standpoint, the district embraces 20 villages and 80 hamlets. In Ethiopia, the Regional States function similarly to states in the United States, with regions further divided into zones, which are then organized into districts. Each district consists of villages, referred to as 'Kebeles' in Amharic and 'Tabias' in Tigrigna. The smallest administrative unit is a hamlet, known as 'Kushet' in Tigrigna, which collectively forms villages. With the potential for agriculture and pasture growth, the area features several seasonal and permanent rivers. It recruited 113 different types of exclosures as of 2018 covering 12.62% of the total land area of the district. Each hamlet contains seven exclosures on average, ranging in size from 1.38% to 51.62% of the total land area.

The average annual rainfall varies between 580 to 750 mm, typically erratic and poor distribution in space and time. The average temperatures range from 18 to 24°C. The climate is favourable for major crops like sorghum, maize, teff, finger millet, sesame, groundnut and some spices and vegetables. Fruits like orange, mango, lemon, tangerine, banana, and java grow well. It has tree species vegetation composed of *Terminalia brownie*, *Acacia senegal*, *Acacia lahai*, *Ziziphus spina-christi*, *Dichrostachys cinerea*, *Balanites aegyptica*, *Ximenia americana*, *Cordia africana*, *Cordia monoica*, *Cordia uncinulata*, *Acacia oerfota* and *Albiza amara*. Vertisols, Aridisol and Alfisols dominate the soils of the district. According to the district agricultural Office, the soil textural types are silt, loam, sandy, clay and silt loam, in order of area coverage.

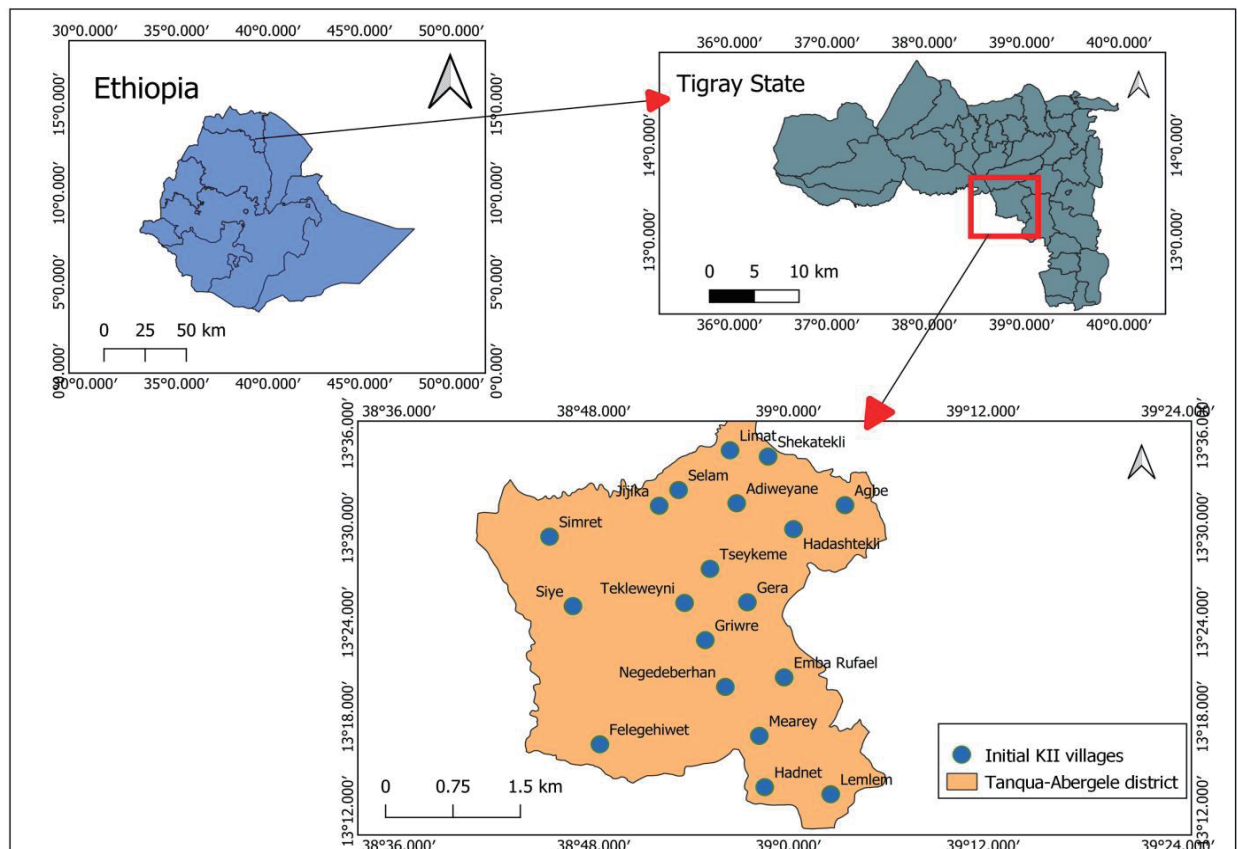


Figure 1. Location of study area (Tanqua-Abergele) and its relative location (region, district and villages) where KII was held to understand characteristics and situation of enclosures.

The total livestock population of the district is 268,266, equivalent to 73,430 Tropical Livestock Units (TLU), of which 116,744 are small ruminants. The district is endowed with a large area of grazing as a result 40-60% of the people make a living on livestock production. The dominant livestock production system is mixed in which sheep, goats and cattle are either seasonally or daily moved in search of feed and water [10]. Tanqua Abergele district is exceptionally known for its livestock population and some farmers own up to 500 livestock heads. Export-oriented slaughterhouse has been established by Abergele International Livestock Development PLC to utilize this endowment through export oriented economic development. Ruminant animals were typically acquired from local farmers and were fattened for an average duration of three months using grass feeding and nutritional supplements. Those animals that met export standards were processed for slaughter by the company in Mekelle, the capital of the Tigray Regional State. The hides and skins were then utilized for leather production at a sister company known as Sheba Leather Factory, situated 45 kilometres from Mekelle. The district is inhabited by a total human population of 92,888 with 51% female and 49% male, with an annual growth rate of 2.7%.

2.2 Data Collection and analysis

Tanqua-Abergele was systematically selected for this study being one of the districts in which the Sustainable

Land Management Programme (SLMP) has been implemented by the government since 2008. A total inventory of all exclosures established before 2019 was conducted in 19 villages of Tanqua Abergele district. Data was collected from Focus Group Discussions (FGD), 76 Key Informant Interviews (KII) and content analysis of documents, project plans and reports. Descriptive statistics (averages and percentages) were used to analyze data. Data was analyzed using STATA 16 and presented in tables and graphs.

3. Results

3.1. Temporal and Spatial Distribution of Exclosure Types in Tanqua-Abergele District

The exclosures established in the Tigray region were broadly categorized into three. Natural regeneration exclosures (ENR) would be the first portion. These areas were solely shielded from animal and human influence, such as tree cutting, grazing and other human activities. It was expected that repressed plants and seeds and seeds within soil seed banks would spontaneously re-grow in this type of exclosure without the need for any other extra interventions. This approach is useful for an instant action because it is a cost-effective and speedy way to establish an exclosure to meet some defined objectives. The second type of exclosure demands the construction of Soil and Water Conservation Structures (SWCS) to halt severe erosion in addition to protection from human and animal interference (ECNR). A third type of exclosure was a practice of relatively integrated management including construction of SWCS, plantation with nursery-raised seedlings and protection from human and animal interference (ECP). As a result, we calculated the percentage of exclosure types in the Tanqua-Abergele district by area covered (ha), and found that 30%, 20%, and 50%, respectively, come under ENR, ECNR, and ECP (Fig. 3 (a)).

We also looked into whether each exclosure was selected to achieve particular community objectives. The detailed reactions of the Key Informants are presented in Table 2. According to KIs, the largest proportion (54%) of the exclosures was established to comply with the national and regional exclosure development plans in a quota system. The second largest proportion of exclosures (34%) was established targeting local development programs like grass for animals and thatching, protection of economically important plant species, development of spring water and healing farmlands by treating erosion-induced gullies. Exclosure areas with a share of 11% were established to buffer some adverse effects on different community resources including rivers adjacent to farmlands, protect grazing by herds from another village, protect churches and tombs, buffer settlement areas and farmlands from upstream flooding and proof of ownership. A small part (approx. 1%) was assigned for private fattening through formal investment and research works on agriculture and natural resources thematic areas.

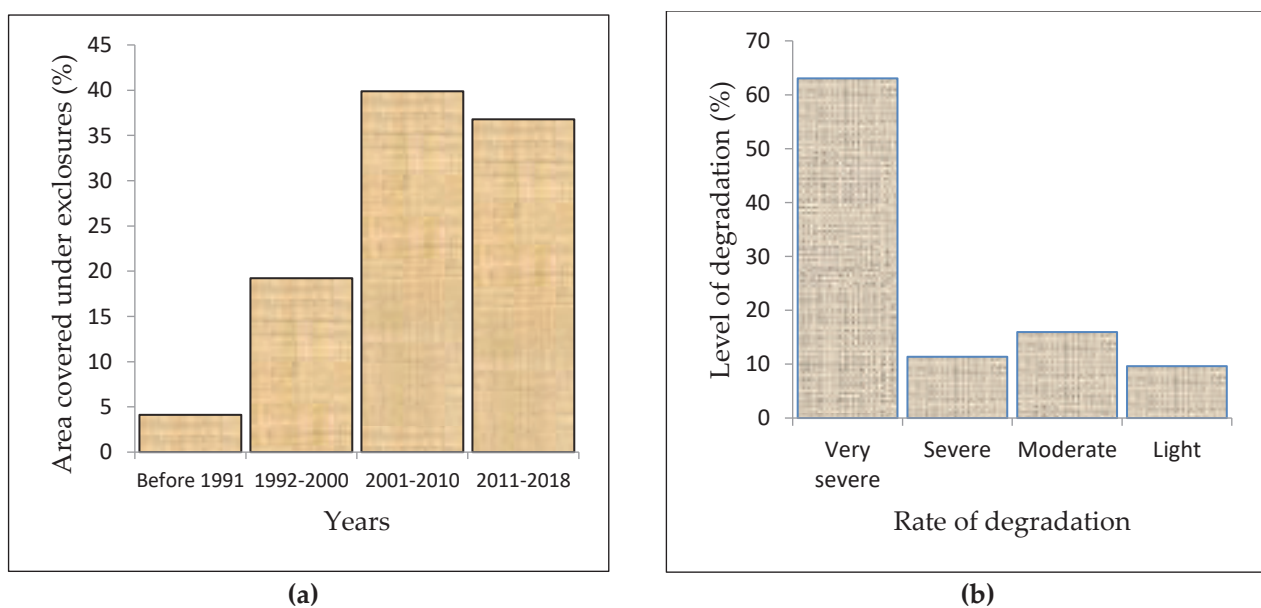


Figure 2. (a) Exclosure Establishment; (b) level of degradation during recruitment of exclosures in Tanqua-Abergele district, compiled from key informants.

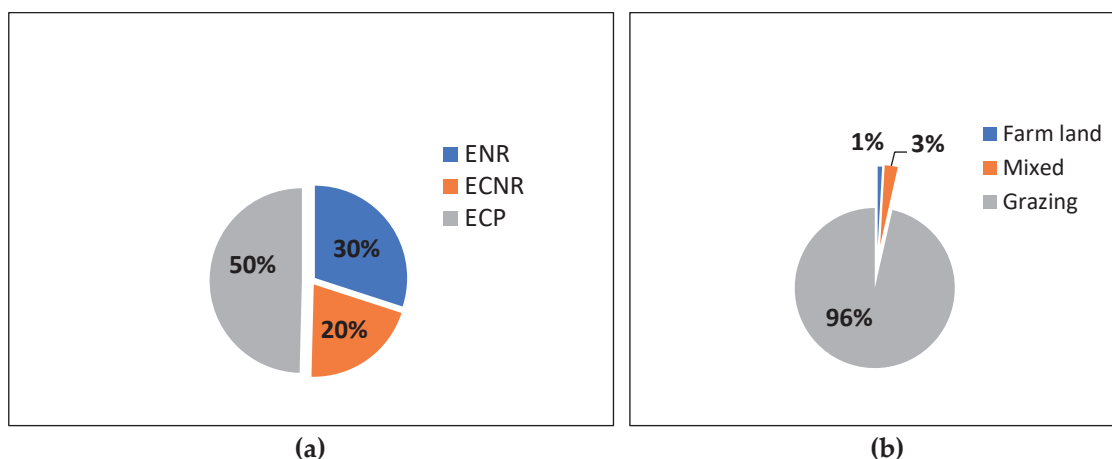


Figure 3. (a) Type of Regeneration; (b) land use of enclosures during establishment in Tanqua-Abergele district of Tigray, compiled from Key Informants.

The highest proportion of the enclosures (96%) was established on communal grazing lands, 2.6% on mixed areas and only 0.9% on farmlands (Fig. 3 (b)). The level of degradation at the time of enclosure establishment was light (9.63%) with only surface sheet erosion, moderate (15.96%) where rills were seen, severe (11.37%) in which small gullies and exposed plant roots were visible while the rest (63.04%) was very severe in which big gullies and landslips were observed. Enclosures intervened with Soil and Water Conservation Structures took 2.58 years on average with a maximum reaching 8 years to be completed. Similarly, the frequency of plantation in enclosures was 5.58 years on average with the maximum being 28 years.

Table 2. Response of KIs on objective of enclosure establishment in Tanqua-Abergele district of Tigray

Purpose of establishment	Number of sites*		Area	
	N		Ha	%
Compliance to Regional Plan	66		9453.41	54.39
Grass, Water and Farmland development	36		5946.12	34.21
Buffer and Protect Community Resources	9		1842.48	10.6
Research and Investment	2		140	0.81
Grand total	113		17382.01	

*Land areas characterized by a single type of regeneration are referred to as sites. A site may be classified as ENR, ECNR, or ECP.

Long-term exclusion of animals and human interventions from protected areas can increase springwater development especially when the establishment objectively considers such factors. We collected information on the availability of command areas that can be described as main economic development zones through crop and animal farming (Table 3). Accordingly, 79 sites have farmlands, 20 sites have no command areas and 9 sites immediate drain to rivers without crossing level grounds. During the data collection period, 52 sites were being used as rain-fed crop production areas, and eight sites had a few scattered fruit trees. Moreover, integrated farming had been undertaken in eight sites and some scattered patches of irrigation plots were observed in 12 sites, targeting subsistence vegetable production.

Table 3. Type, size and current use of enclosure command areas, Tanqua-Abergele district of Tigray, Northern Ethiopia

Type of command area	Command Area		Size of command area			Current use of command area		
	N	A(%)	Size	N	A(%)	Use	N	A(%)
Farmland	79	69.91	***	4	3.54	F	52	46.02
Farm and Grazing	5	4.42	**	68	60.17	FG	12	3.54
No command area	20	17.7	NA	29	25.66	FGI	8	7.08
Flow to a River	9	7.96	*	12	10.62	FSI	12	10.62
						NA	29	25.66
Total	113	100		113	100		113	100

N (number of sites); A (size of the command area); * (Small), ** (Medium), *** (Large); NA (Not Applicable as there is no defined command area); F, FG, FGI and FSI represent Farmland, Farmland and Grazing, Farmland and Good Irrigation, Farmland and Small Irrigation.

Enclosures linked with other neighbouring regions, districts and/or villages were five, 16 and 54 sites, respectively (Table 4). We asked if enclosure-connected administrative units cooperate to construct SWC structures and protect enclosures from any illegal acts. Unfortunately, only five sites have contributions from the bordering administrative units in the development and management of enclosures. According to respondents 63.73 and 59.19% of the enclosures were challenged by illegal grazing and tree cutting while 17.15% triggered conflicts among neighbouring community members. Illegal and continuous tree cutting and grazing have been serious problems facing enclosures, which ultimately resulted in the abandonment of many enclosures. Accordingly, the district established 24,304.23 hectares of enclosures but 7,003 (29%) were not recognized as enclosures by the local administration. More worryingly, there were enclosure areas whose restoration outputs primarily benefit nearby villages. Conflicts were noticed in these situations where use and management rights were under different bodies.

3.2. Enclosures objective, illegal grazing and tree cutting and Conflicts

According to KIs, 64% and 59% of the enclosures were challenged by illegal grazing and tree cutting while 17% triggered conflicts among community members. We found out that the setting of objectives at the time of establishment was not participatory. If community members were fully engaged, they could have prioritized other effective enclosures in terms of suitability for ecological and economic development.

Table 4. Response of KIs (Resp.) on meeting of enclosure objectives and presence of illegal acts, administrative link and conflicts in terms of area coverage (%) and number of sites (N) in Tanqua-Abergele District of Tigray, Northern Ethiopia

Resp.	Illegal Grazing		Illegal Tree Cutting		Link			LAU WT*	Conflicts	
	A	N*	A	N*	Reg.	Dis.	Vi.		A	N
No	36	47	41	50	108	97	59	65	83	100
Yes	64	66	59	63	5	16	54	5	17	13
Total	100	113	100	113	113	113	113	70	100	113

* LAUWT if linked units (Region, district or village) work together (Note 43 enclosure sites are not applicable as they were not linked to other administrative units)

4. Discussion

4.1. Temporal and Spatial Distribution of Enclosure Types in Tanqua-Abergele District

It was revealed that a miniature of land was covered by enclosures before 1991 when majority of the Tigray region had been governed by Tigray People’s Liberation Front (TPLF) [11]. On community grazing lands, nevertheless, a series of expanding enclosure establishments occurred after that period (Fig. 2 (a)). Similar to the current study [3] and Yami et al. [12] reported an increase in the extent of the enclosures in Tigray following the downfall of the

Derg regime, which ruled Ethiopia from 1974 to 1991. The enclosure establishment peaked between 2000 and 2010, although it started to decline gradually by 2018 (Figure 2 (a)) due to constraints related to enclosure governance and law enforcement [13]. Farmers indicated that the general trend of enclosure establishment has not continued at the pace it started and swapping of a particular area between enclosure and grazing land has been a phenomenal occurrence.

Currently, 12.62% of the district's average land area is enclosed. There are variations among villages with the village of Giriwre having the lowest (1.38%) while Hadnet has the most (51.62%) coverage. This is in agreement with [3] who reported that the region's enclosure showed a wider variation between one and seven hundred hectares. Moreover, Mekuria and Aynekulu [8] found 3-16% of enclosure coverage in their study in four districts of Tigray. The significant differences in the extent of restored areas across various administrative units suggest that the establishment of enclosures continues to adhere to a quota system implemented from the top down, and has not yet been firmly established as a systematic practice within villages. Had villages been empowered and restoration been an agreed goal, figures would have been consistent among villages and time horizon. This further explains the reason for sustainability challenges facing those already established enclosures. Top-down implementation and management of restored areas can compromise conservation efforts [14] and are usually not effective [15]. The top-down approach still dominates in Africa although some examples of translations to local government [16] were reported. Similarly, local government empowerments were communicated in China [17]. However, there is much practical evidence that the top-down approach is still a dominant system in Ethiopia [18-22] negatively affects restoration regimes. The bottom-up system was reported as a better social-ecological fit [23] as an outcome of their study on restored river governance. Bottom-up planning, implementation and evaluation were also reported as successful in Brazil [24]. A bottom-up and top-down integrated restoration planning approach was recommended by [25] who reviewed different papers. Although considerations were not taken into account by the regional authority, communities at the grassroots level attempted to link certain criteria for selecting specific sites for forest development after quotas were submitted within the framework of a top-down approach. Inherent top-down and bottom-up integration arose, which necessitated standardization of the existing system.

The three categories of enclosures, ENR, ECNR, and ECP, have been measured to cover areas proportionally at 30%, 20.4%, and 49.6%, respectively. There is a large variation between villages in terms of the type of enclosure established on degraded community lands. Often, ECNR develops inadvertently during times of shortage of seedlings to plant and seeds to sow, leaving some areas bare after the construction of soil and water conservation facilities. Restoration schemes can be generally established as natural or artificial [26, 27] regeneration. At Tigray, we note a distinctly broad category of three enclosures. The first is the natural regeneration of vacant lands that are protected only from human and animal interference such as the felling of trees, movement of people inside and grazing (ENR). The establishment of this typology assumes that the ENR can benefit from the regenerative potential of naturally available suppressed plant species and the soil seed banks of protected parent plants without further intervention. It is economically feasible and very useful when immediate local action is necessary to enhance vegetation [28, 29]. The second type of enclosure (ECNR) allows for the construction of soil and water conservation structures (SWCS) to prevent severe erosion in addition to ENR interventions. The third type of enclosure is a management practice that combines the planting of seedlings and enrichment with different plant seeds of the ECNR resulting in another type of forest hereafter referred to as ECP. The three methods of forest regeneration are expected to differ in ecological and socioeconomic impacts. A meta-analysis of 133 studies by Crouzeilles et al. [30] confirms that natural regeneration surpasses active restoration in biodiversity and vegetation structure. A similar report [31] indicated that natural regeneration has the potential to be better for forest regeneration, biodiversity and carbon storage than active restoration. Evans et al. [32] revealed that natural regeneration is twice as likely to sequester carbon as tree plantation in Queensland. Moreover, Holl [33] recommended natural regeneration as the best approach to restoring tropical forests. The aforementioned evidence affirms that natural and active restoration approaches are expected to diverge in their ecological and economic impacts. Research and development practices can focus on the selection of appropriate sites, regeneration approaches and spatial distribution of enclosures which are in harmony with the potential for ecological and socioeconomic development.

4.2. Objective of enclosure establishment

A more thorough evaluation of each chosen area's compliance with specific community goals and whether those objectives were compatible with the social, economic, and environmental advancement of the local populace revealed interesting outcomes (Table 2). Surprisingly, most enclosures were created to fit national and regional forest development plans under the quota system. This implies that the focus was on conservation with no clear goals on other sustainability pillars such as economic and social benefits for local people, given the priority of regional and national governments is to address degradation [34]. This further confirms the popularity of the top-down approach in the region. Members of the local community who were interviewed said that the top-down method to establishing enclosures wastes a lot of resources since there was no proper and participatory site selection, did not meet the aims of the community and failed to take strong protective measures. More often than not, enclosures are susceptible to partial grazing, destruction of soil and water conservation structures, illegal harvesting of produce, or complete destruction and conversion to grazing. As enclosure quotas [19] are either accompanied by food aid [35] to integrate environmental and local development or administratively decided figures [19], villages are mostly engaged in a vicious circle of deforestation ↔ reestablishing ↔ deforestation. Illegal grazing [36, 37] and tree cutting [35] were reportedly mentioned to have adverse effects on the vegetation and soil [36]. Studies that reportedly proved the success stories including improvement in soil chemical and physical properties [38], vegetation parameters and water development [39-41] on enclosure than adjacent grazing areas are outcomes of avoiding some level of illegal grazing and tree cutting [42]. Reversing enclosures back to illicit and unplanned grazing and tree cutting is just enabling the drivers of environmental resource depletion into action. Therefore, enclosures established under the regional premises and motivations of conservation to address degradation issues alone were unsuccessful.

The second largest proportion of enclosures was established targeting local development programs like grass for animals and thatching, protection of economically important plant species, development of spring water and healing farmlands by treating erosion-induced gullies. This category benefits from interventions from NGOs and is usually organized under the concept of watershed management, the potential for targeted woody plant species and grass. Construction of important SWC structures, hiring of guards, and attempting to connect to the command areas are key features that make their fate better than other categories. Important problems affecting the sustainability of these enclosures are unplanned and sudden withdrawal of guards and handover to the community without a well-organized system for sustainability. Weak rules were proved to affect the sustainability of enclosures in Tigray [13]. Under this category is also the protection of economically important plant species like *Boswellia Papyrifera* [43]. Maintaining key management features such as guarding until conservation and establishment objectives are attained, would help sustainability. Identification of community problems and community-driven solutions can enhance the ecological and socioeconomic contribution of enclosures.

Thirdly, some enclosure areas were established to buffer some adverse effects on different community resources. Administrative restructuring of villages to suit development interventions and governance resulted in ownership problems of commonly used grazing areas and the village that claims ownership decided to protect them by changing into enclosures and assigning guards. Moreover, when a grazing area of a village is adjacent to another village it decides to buffer its grazing land by establishing an enclosure bufferzone of a few kilometres. These types of enclosures are community-driven and are emerging opportunities for sustainable enclosure development. However, the goal must be in line with the economic and ecological development plans of the village that owns the forest area, and the systems must be such that conflicts typical of forest programs do not arise. Communities may aim to designate certain areas as buffer zones; however, these areas often evolve into natural forests that connect with other designated enclosures, thereby improving forest management initiatives. A study conducted by [44] corroborated these observations.

Other community motivations for establishing enclosures including protection from the effects of flooding on farmlands along riverbanks, settlements, churches, and sacred cemeteries, are relatively sustainable. When local community objectives for establishing enclosures are to protect farmlands from prevailing torrential rains, Drbal et al. [45] suggest identification of critical points to curtail the risks of flood hazards is useful. Physical structures reduce runoff and allow nutrients to be absorbed by protected soils, improving vegetation on degraded fields and further boosting agricultural protection [46]. In practice, integrated landscape management protects lands

downstream that may be settled or used for farming. However, as plans are not created in conjunction with stakeholders, localities lack the capacity to design comprehensive and integrated solutions that cover the economic, social, and protection spheres. This calls for the establishment of grassroots institutions that should be periodically equipped with financial, physical, and human resources.

A small proportion of exclosures was established for private fattening PLCs and Research works on agriculture and natural resources development. These establishments have little support from the general public, are frequently targets of theft and illegal grazing, and are only safeguarded by tight guarding procedures. In theory, such investments could garner public support through job creation, free community services, and technology demonstrations. Investment is a good starting point to increase private sector participation that is currently very limited, but urgently needed to scale-up and accelerate landscape restoration [47]. When fully involved, the private sector will function effectively, as evidenced by the success of tree planting in China [48]. Designated research areas can be helpful to identify limitations to successful degraded land restoration and measure restoration success, assess the impact of disturbance management, trends and suggest future research directions [49]. However, grassroots participatory planning is useful but lacking [13].

4.3. Pre-exclosure land use

Most of the exclosures (96%) were established on communal grazing lands, which significantly reduced their size. This report is in agreement with Mekuria [1] and Napier and Desta [50]. The shrinkage of communal grazing lands [51] promoted overexploitation [52] exposing it to degradation. This implies that the recruitment of exclosures is done according to the geographical location of the grassland without focusing on the suitability of the land for economic and ecological development. Under the 4-returns, 3-zones and 20 years approach [47], a natural zone with forest and nature restoration; a combined zone with mixed agriculture and nature; and an economic zone with sustainable production need to be identified. In the current study, the combined and economic zones are missing which challenges its sustainability. The tangible benefits of exclosures to household income in the study area were found to be meager [53]. Therefore, the economic and ecological suitability requires re-evaluation.

4.4. Level of Degradation, Duration of SWC and Plantation of exclosures during establishment

Although the establishment process did not take into account the degree of degradation analysis and therefore, no specific recommendations were made, our discussion revealed that 63% were very severely degraded at the time of inception (Figure 2 (b)). To help understand the soil ecosystem during forestation, respondents were asked to list degraded lands under the severely degraded categories if it was highly dominated by gullies and landslips. Indeed, 75% of the areas selected for reforestation were severely and very severely degraded at the time of inception. Therefore, most forests are intrinsically justified in terms of protecting soil from erosion. The level of degradation at the time of establishment has a direct effect on the duration of restoration [54]. The planning, implementation and assessment of restoration of degraded land had to be based on recommendations, which in turn depended on the severity of the degradation. However, the quota system did not come up with specific recommendations for restoration as it did not even properly categorize the areas depending on the severity of degradation. However, landscape restoration cannot follow a one-size-fits-all solution, but depends on complex socio-ecological systems that bring challenges and opportunities that can best be answered through a framework program for planning, designing, directing and monitoring systemic reforestation projects [55]. One such foundation documents basic information about the severity of degradation [56] and the overall suitability of the land for environmental and economic development. Severe degradation for example, extends the payback period of restoration [56]. In this study, soil and water conservation (SWC) structures at closed sites took an average of 2.58 years to complete, and in some cases the maximum time was 8 years. Similarly, exclosure plantations are conducted annually for 5.58 years and the maximum length is 28 years. The good news is that a comprehensive approach to restoring degraded lands and maintaining their ecological and socio-economic sustainability is possible by conducting a preliminary analysis of land suitability for economic and ecological development. Scientific evidence shows that degraded rangelands lose resilience through loss of resistance rather than loss of recovery potential [57].

4.5. Command Area, Cooperation and Conflicts

Our assessment of the results, where 79 of the enclosures flow into farmland, nine into rivers, and 20 have no command area, indicates that little consideration has been given to the suitability of the land for economic and ecological development (Table 3). In addition, some have less potential to either improve groundwater renewal or lack a well-thought-out water use strategy. As a result, of the enclosures with command areas, 52 sites are used as rain-fed cultivation areas, where scanty irrigation is tried, for example, fruit trees and vegetables are grown. The availability of command areas is linked to an economic development zone through crop and animal farming [47]. As a direct effect of enclosure, especially if a new source of water is created due to groundwater recharge, enclosures can be considered very suitable for strong economic and ecological development.

Enclosures linked with other regions, districts and villages are 5, 16 and 54 sites respectively. We asked if linked administrative units cooperate to construct SWC structures and protect enclosures from any illegal acts. Unfortunately, only 5 sites have contributions from linking administrative units in developing enclosures. Moreover, there are sites whose success only benefits mainly another adjacent village. In such cases, conflicts were observed.

5. Conclusions

Even if the motive of the regional administration was to conserve, communities have taken some steps and attempted to establish enclosures based on different common interests (buffer, grass, church protection, secure ownership, occasional grazing during worse conditions, water development, etc). This gives a hint that decentralization and community empowerment can lead to objective-based enclosure establishment. Moreover, communities have different problems, desires and expectations from delineating a particular area as an enclosure and thus blanket recommendation of conservation cannot work as a blueprint for all villages and districts. Local community demand-based enclosure establishments are likely to be sustainable as defined objectives can be met by the specific enclosures recruited. We conclude that a pre-establishment assessment was not properly conducted which is critical for successful enclosure development and decision on type of enclosures, their management and objective evaluation of progress. Based on well-established appropriate criteria, enclosure must be introduced as an ecological restoration and economic development strategy. It is essential to research the economic benefits that communally used grazing lands already provide to nearby people and to create enclosures that may effectively provide those benefits. This may encourage farmers to sign up for the program and voluntarily seek out other degraded communal lands that would require more time to provide economic and ecological benefits

We recommend a detailed characterization of future recruitment of degraded land for restoration programs, considering that the land is suitable for the local population's sustainable ecological and socioeconomic development. Community members should discuss each newly selected area for enrollment to enclosures and detailed records should be kept for monitoring and evaluation. To do these strong institutional arrangements at the village level is crucial. Clear guidelines on planning, implementation and evaluation of enclosures are required. In the case of regions, districts and villages are found to be connected by enclosures, participatory planning, implementation and evaluations are important.

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