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Water and the Future of the Middle East & North Africa (MENA)

Michael Brett-Crowther *

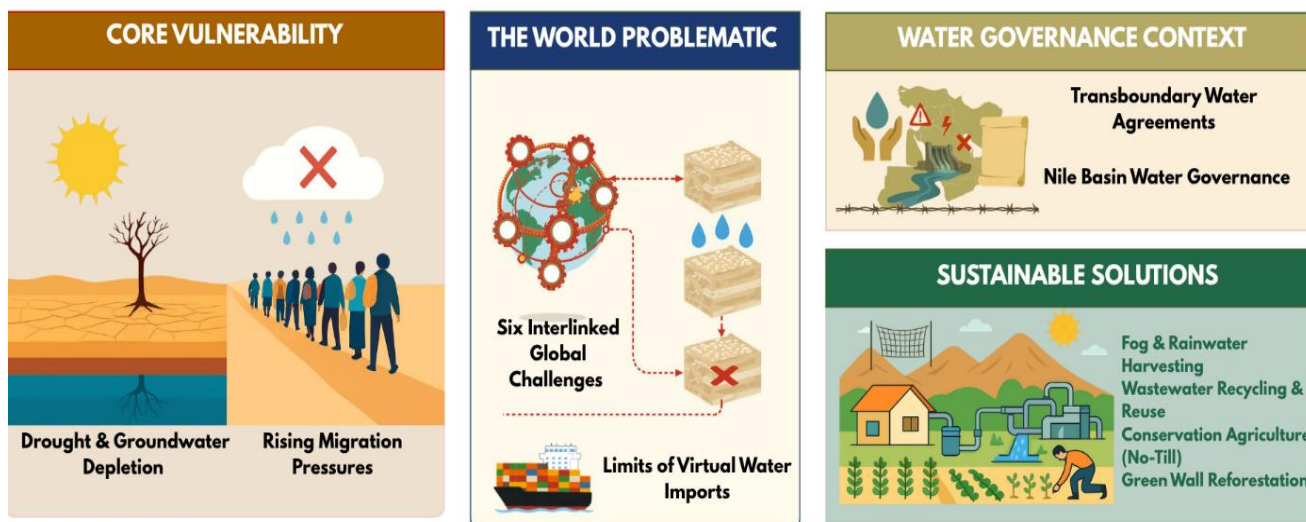
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Graphical Abstract

Water stands at the centre of the “world problematic”, where food security, migration, conflict, energy, and ecological decline converge within an increasingly fragile global system. Across the Middle East and North Africa, geopolitical rivalries, demographic pressures, and technocratic ambitions have intensified water scarcity, even where technical solutions already exist. Conservation, recycling, rainwater harvesting, wastewater reuse, and regional cooperation are essential responses.



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Abstract: Water is central in the “world problematic”, linking food, energy, poverty, migration, conflict, and development within a deeply interconnected ecological system. The water crises affecting the Middle East and North Africa merge with geopolitical tensions, national ambitions, demographic pressures, and the technocratic planning that accompanied dam policies and agricultural expansion. Examples such as the Nile and the Tigris and Euphrates, Morocco, Ukraine, the African Green Wall, and major hydraulic projects in Ghana and Ethiopia show that technical solutions often exist, yet fail because of political inaction, economic interests, and the absence of genuine cooperation. There is a need for new forms of conservation, recycling, and water reuse — including wastewater recovery, ablution water reuse, drip irrigation, fog harvesting, and rainwater collection — in order to adapt societies to water scarcity. If governments continue to ignore the interdependence between environment, justice, and human survival, ecological disasters, mass migration, and conflicts over water may become the normal condition of the future world.

Keywords: Water scarcity, MENA region, Water governance.

Water and the future of the Middle East & North Africa (MENA)

“ ‘Ecology’, wrote Sir Julian Huxley, ‘is the science of interrelations. It studies the balance to be achieved in a system of interacting factors.’ It is, in fact, essentially a dynamic subject, and it is ultimately resolvable into the role of the individual organism in these cycles of production and breakdown that characterize the ecosystem - cycles which may be seen to group themselves under three headings - energy, nutrients and water. A dynamic approach to ecology provides a framework on which it is possible to hang the unifying principles which bring together its various branches” [1]. Black also notes: ‘Man is, indeed, in the centre of the ecological system, and whatever he does alters the delicate balance of the components and reacts upon himself [1, p. 5]

Every living being needs water. The Holy Quran states that all life began in water (Surah An-Nur - 45) (24:45). Water makes up at least 60 percent of the human body. Water is sometimes defined as being a basic human right. In MENA, water is a vulnerable resource because precipitation is often irregular and unevenly distributed, groundwater reserves are depleted, rivers are not abundant, drought is a common experience, and so living conditions for many are poor. One result is pressure to leave the affected area.

At the sixth special session of the U.N. General Assembly, Plenary Meeting 2207, 9 April 1974, Kurt Waldheim, then Secretary General, U.N., defined six primary problems: food, energy, population, mass poverty, military expenditure, world monetary system [2]. These we regard as constituting the world problematic. A problematic – *problématique* (Fr); *problematik* (Ger) - is a multi-problem; its interactions offer solutions. This idea appears notably in a lecture by Aldous Huxley in 1963 [3] and two books by C.H. Waddington [4,5].

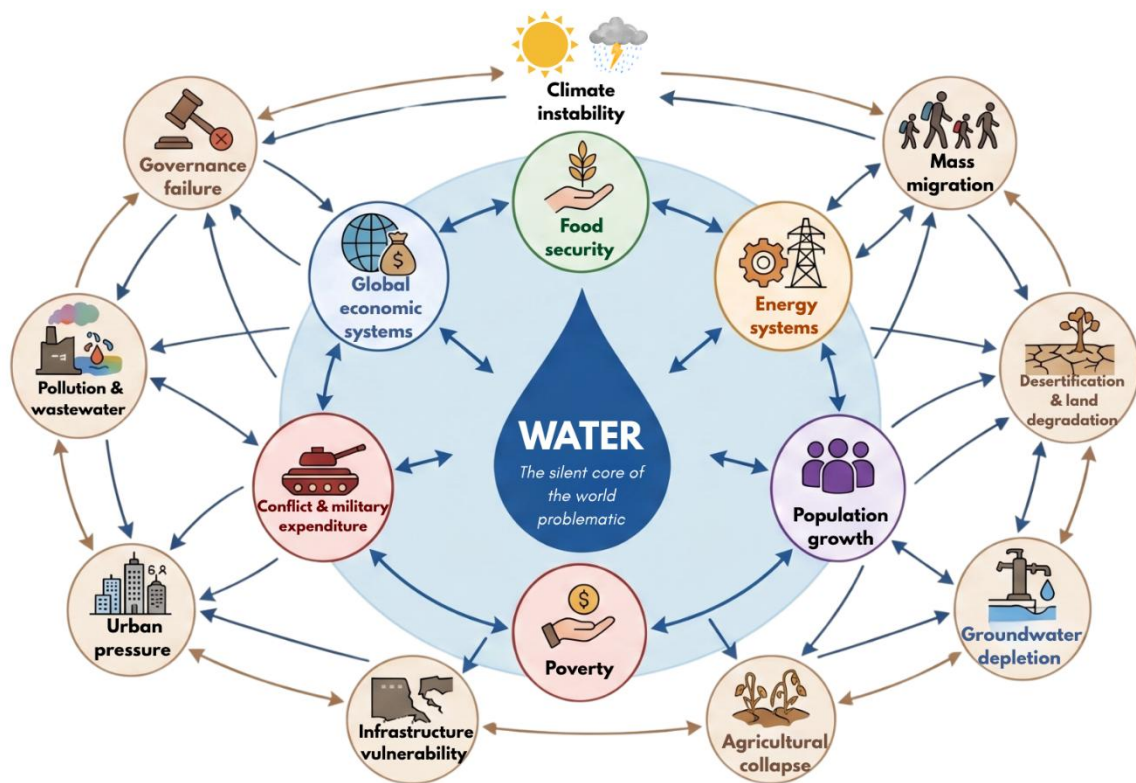


Figure 1. Water at the centre of the world problematic

The world problematic, or the world multi-problem, is the six primary problems. These problems all concern water. How can they not? Briefly, the major crops of rice and wheat and herds of livestock depend on water. Energy systems include hydro-electricity and water for coolant in nuclear power plants. Populations need water for hydration, cooking and sanitation. Mass poverty is accompanied by a water shortage or the presence of floodwaters. Military expenditure tends to equal or exceed the costs of development, and development always requires and affects water. The world monetary system since 1945 has been dominated by the US dollar, and through the World Bank and the International Monetary Fund it has been central to water-related development in many countries. The world monetary system is now subject to gigantic forces of speculation, cryptocurrency movement, and the games of billionaires.

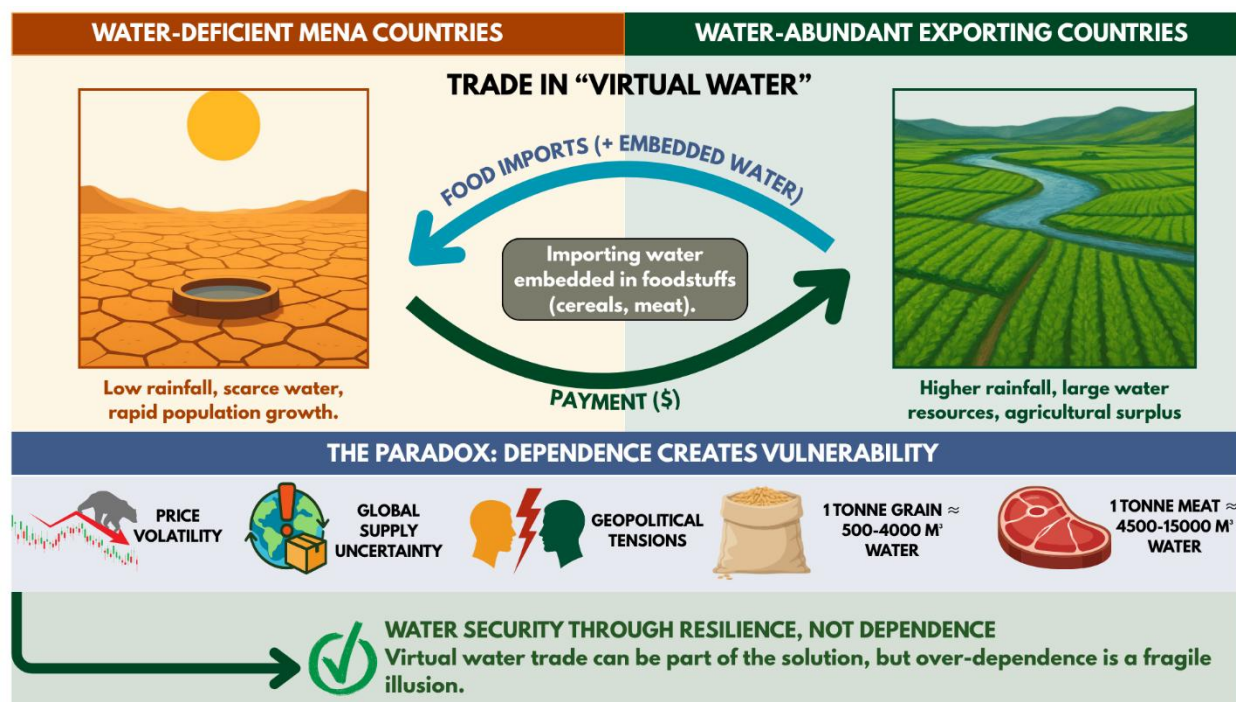


Figure 2. The paradox of virtual water dependence in MENA

There is another dynamic relation to be considered, proposed by J.A. Allan in 2009 [6], that of virtual or embedded water in regard to these problems. This idea of virtual or embedded water means that water-deficient areas overcome their problem by importing water in the form of the foodstuffs which have been grown using water in water-abundant countries. Thus, disputes about water - because disputes arise over transboundary flows or restrictions of flow in regard to rivers - should be unnecessary. The idea concerns most imports of cereals. The difficulty is that the idea of embedded water collides with some hard facts. Foreign exchange may not be adequate in some poor countries. The amounts of water needed to produce goods for trade may be very great; e.g., it takes 500-4000 tonnes of water to grow a tonne of grain, depending on location and the maximum difficulty is in MENA, and 4500 to 15 000 tonnes of water to produce a tonne of meat (personal communication, David Dent, January 3 2026). It is not certain that the producer countries will continue to have abundant water. In addition, MENA has urban centres and well established rural centres. It is culturally normal to have a family farm and to work it in a traditional way. So, there is resistance to depending on outside sources, although many MENA countries do. The problem is like persuading village India to give up cooking with cowpats to depend on paraffin or bottled gas. More alarmingly, if one thinks of emergencies, one thinks of famines in several countries at once and the difficulty of supplying their need with imports of grain from the US, where stockpiles are not certain to be adequate. And the U.S. has its own problem of reduced water availability; e.g., in

California. If Muslim, anti-communist Indonesia had a famine at the same time as capitalist but also communist China, or Muslim Pakistan and Hindu India, who would be given food grains and or feed grains first? The fact of human nature and actual cultural patterns is a harder fact than that of virtual water. Further, US politics do not encourage belief in reliable responses to the world outside the US.

Houari Boumedienne made some telling points in an interview with *The Times* in 1974:

‘With Europe we could start to build up a new kind of world society, with more human and spiritual values, in which human beings can find true fulfilment, and where poverty and misery can be eliminated. Together we could seek a new life-style which will make possible the sustenance of the 8,000 million people expected on this planet by the year 2,000. Otherwise, no number of atom bombs will stop the tidal wave of billions of human beings who will one day break out of the poor south of the world into the relatively open spaces of the rich north in search of survival [7].’ This prospect of world war was clear to Boumedienne, and today, in 2026, it seems still to be a great danger.

Boumedienne was right. Various problems have been pushing people out of their homes for many decades. There is migration into Europe from South of the Sahel and from MENA. Boumedienne's hope for ‘a new kind of world society, with more human and spiritual values’ is integral to the European Union. But we must not ignore the opposite effects of world politics and human aggression. A century ago, world politics included ‘the scramble for Africa’, whereas now after all the changes, China and the United States and Saudi Arabia seem to reverse the trend. Africa is being re-colonized. The toxicity of water in Zambia with depleted fishing stocks appears to be related to Chinese management of copper mines [8]. The luxury of the Gulf States complicates assessment. Their carbon footprint contradicts their innovations in solar energy and desalination (distillation of seawater).

The Global Challenges Foundation has produced a report on Global Catastrophic Risks 2026. This identifies 4 areas: Catastrophic Global Change, Ecological Collapse, Weapons of Mass Destruction, and Near Earth Asteroids [9]. All predictions are meant to provoke action in the immediate situation, because the willingness to prepare for a future event depends on many variables, and options are always being reduced by the passage of time. Talking about a tipping point, or leverage, or governance, or justice does not cause action to follow. These analyses do not appear in the ordinary newspapers, or most radio or television programs. It is also necessary to say that rational thinking is frequently absent. The person who knows that water pollution can be reduced is not the same as the person who may, e.g., control the tanning factory using chemicals

which are discharged to the river; and he may have no interest at all in maintaining any system to reduce those chemicals or to separate them from the receiving water body. If someone refuses to pay for his pollution, the principle that the Polluter Pays must be enforced. How can the law be enforced when the legal system is compromised? Many countries show this problem; including the US.

The great achievement of Boumedienne, the green wall against the desert, remains a resource which can be used and can be replicated. Trees were planted by Boumedienne using armed soldiers to ensure that they were protected as they became established. Enforceable policies for water, conservation, and mitigating the effects of climate change will be necessary if global challenges do not produce global actions. The African Union's attempt to replicate the successful green wall appears to have failed, or at least to be interrupted, delayed, incomplete [10]. This shows us that we must either cooperate or be coerced. If we do not willingly, with real cooperation, attempt to solve the six primary problems - the interlinked problems of the environment - we may have to be coerced by governments or compelled by necessity. That necessary compulsion will probably be an accumulation of catastrophes.



Figure 3. The evolution of the Green Wall concept

The current division of Libya into two regimes does not appear to be important to the EU, the AU, the US, the GCC. Divided Libya does not evoke any coercive response or any emphatic call for

cooperation. We may ignore data about environmental problems merely because everything else seems to be tolerable - or unimportant. Yet expansion of deforested areas of the Amazon, mounting evidence of the presence of pollutant plastic in the ocean, even at its deepest point - the Mariana Trench - and the apparent worldwide spread of nanoplastics even in Pakistan's salt should require decisive action; and this has been the apparent object of the COPs. But, everything is somehow managed into serious but ineffective words, time and again.

In Russia's invasion of Ukraine in 2022, the Russians seized control of Zaporizhzhia Nuclear Power Station and in 2023 destroyed the Khakivska dam on the river Dniepro (Dnieper). This has not aroused any condemnation from the US. Similarly, the Americans have ignored the Russians' attack on the Chernobyl (Chornobyl) site, and have not condemned Russia for its dangerous activity. Russia's leaders have always controlled the Russian population by repression, and the Kremlin misrepresents Russia's war against Ukraine. If catastrophes at Zaporizhzhia occur, will the Americans take any serious action against the Russians? The American inaction reflects the arbitrary and destructive decision-making of the current administration. Those features reject the idea of the world problematic and the Sustainable Development Goals.

Let us, instead, be realistic and consider some facts. Morocco's food supplies increased because of population growth from 3.5 to 9.5 million, from 1921 to 1954. Rain-fed agriculture was unreliable. The Kassa Tadla scheme in the Umm er Rabia basin was inaugurated in 1935 and by 1945, the reclaimed areas were a complete success. A tributary of the Umm er Rabia was then harnessed, the Oued el Abid. The gorge for this ran at a higher level and could be more easily dammed. When this was finished in 1952, the height - about 222 metres / 400 feet - was the highest in North Africa, and it held 1.5 milliards of water - a greater volume than was held by the Assuan reservoir across the Nile.

Water released from the Ben el Ouidane dam went first to hydro-electricity stations then was diverted north in a tunnel through the dividing ridge between the two rivers; then into the Umm er Rabia valley. From here the water went to the Beni Moussa plain to irrigate excellent land [11].

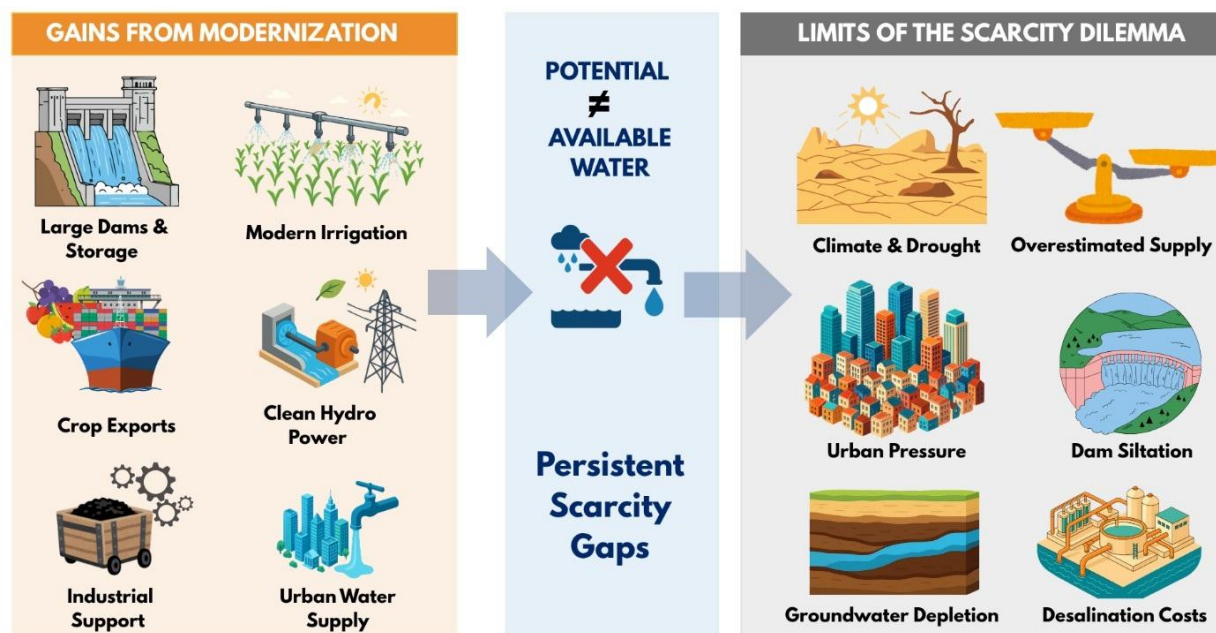


Figure 4. Morocco's hydraulic modernization and the scarcity dilemma

In 1977, it was believed that probably 155,000 hectares were under irrigation in Morocco, and that a further 930,000 hectares could become cultivable if they were irrigated. It was also clear in 1977 that dry-land areas could produce cereals with high-yielding varieties and chemical fertilizers [12]. There has been research in Morocco into the agri-food system, and an excellent monograph edited by Nadia Boutaleb brings many of these matters up to date [13]. Morocco is now a successful exporter of food to the European Union. Her economic development through phosphate exploitation and the development of tourism is impressive.

But Morocco has a water problem, and a population of 38.8 million (2026). For many years, the idea of 'renewable' water or 'exploitable water' and 'hydraulic potential' has been current in regard to Morocco's development. This idea is related to quantification of water in regard to agricultural expansion [14]. The 'dam policy' inaugurated in 1967 aimed to irrigate 1 million ha by the year 2000. One result appears to have been to confuse 'potential' with actual limits, and perhaps also 'potential' has been exaggerated by 50 percent [15, 16]. The water storage capacity increased from 1.5 Bm³ in 1956 to 16 Bm³ in 2000. It is probable that inaccurate figures have crept into the policy making, so that instead of 20–22 Bm³ of available water, there may be in fact only half that volume [16].

The discrepancies among figures and definitions of water (renewable, exploitable, unconventional) mean that the anticipated need for water in Morocco cannot be met from the existing resource base. If desalination (distillation) is chosen, both capital costs and operating cost may be very high. If reuse and recycling of wastewater is applied more widely, this, like distillation, has considerable

costs and requires changes in infrastructure. But there are also benefits. Retrofitting rainwater collecting systems to houses is advisable, which means also attempting small-scale purification of the harvested rainwater (e.g., from apartment blocks). The removal of sediment from dams and its application to fields as a form of soil amendment is also advisable. Collecting fog water is another option in certain areas [17]. Reuse and recycling includes ablution water from mosques in some countries [18]. It is not known whether this has been considered by the authorities in Morocco. There appears to be a debate within Islam on the reuse of water for ablution water [19]. There is certainly a view that *water used for ablution can be reused after ablution has taken place* [18]. This is authorized by *fatwa* in Yemen and in Oman, in Indonesia and Malaysia. A study of the potential for the reuse of ablution water in Morocco may disclose a potential well beyond expectations. The general idea of abundant water may be perhaps set aside for an idea of expanded uses of wastewater. These uses may include sites where circumstances permit the introduction of constructed wetlands [20].

But local development is often questionable. The Volta River Project was established to develop the bauxite resources of Ghana. It began with the damming of a huge area, making the Akosombo Dam the largest by area in the world. No environmental impact assessment was undertaken. After the dam was built, British scientists William Kershaw and Michael Pugh Thomas were brought in to survey the project and assess it (personal communication, 1973). Pugh Thomas was a freshwater biologist, Kershaw a tropical medicine specialist, in trypanosomiasis. These researchers realized that no-one had considered the effects of the scheme beforehand. In the late 1950s, there was no environmental impact assessment.

The American investment in the dam gave the majority benefit to the aluminium producers. The dam produced some electricity for Ghana and neighbouring countries. But the dam buried the best soils under its extensive waters. Fertilizers had to be applied to the remaining available farmland, and eutrophication of the waters in the dam followed.

These matters do not appear in the Central Intelligence Agency's analysis of the Volta River Project, available in a sanitized version on the Internet [21]. Instead, it is clear that Kwame Nkrumah was over-ambitious, was out-manoeuvred by the two American companies who were negotiating for the bauxite, and control of the project, and who produced a low price for the electricity, but did not increase productivity or employment or make the project profitable, for Ghana. The United States gained influence in Africa. Ghana's debt was used to strengthen that influence. China has done the same with its international loans, in the Belt and Road system of expanding world influence.

Similar stories can be found for many big dam projects elsewhere in Africa. At present, there are still echoes coming from the decision by Ethiopia to build the Grand Ethiopian Renaissance Dam (GERD) on the Blue Nile. This will reduce the available water for Egypt and Sudan as a result. GERD may be justified as a producer of electricity, but it is not so obvious that Ethiopia has the capacity to use electricity or the pipework or tanker system to supply mountain settlements. Ethiopia's population is, however, very great (pop 138, 575, 630) and there is a lack of family planning. Nevertheless, these are not reasons for allowing Egypt (pop 120, 012, 870) and Sudan (pop 52, 763, 790) to have the majority of the Nile's water [22, 23].

In a customary phrase, Egypt is the gift of the Nile. In Pharaonic times, huge structures - the Pyramids, various temples, major cities - were constructed from stone moved by vessels along the river and then moved into place. Necessary crops depended on the river - as they still do and its waters were a means of transport for all commodities and resources. In 1929, Great Britain produced a diplomatic Note which stated that the greatest allocation of the Nile's water was for Egypt. Every other country in the river basin 'shared' to a much lesser extent the waters on which they relied. In 1959, Egypt and Sudan made a treaty confirming their shares of the Nile and *showing no concern for any other country*. Yet there are eleven countries within the Nile river basin: Burundi, the Democratic Republic of the Congo, Egypt, Ethiopia, Eritrea, Kenya, Rwanda, Sudan, South Sudan, Tanzania, and Uganda.

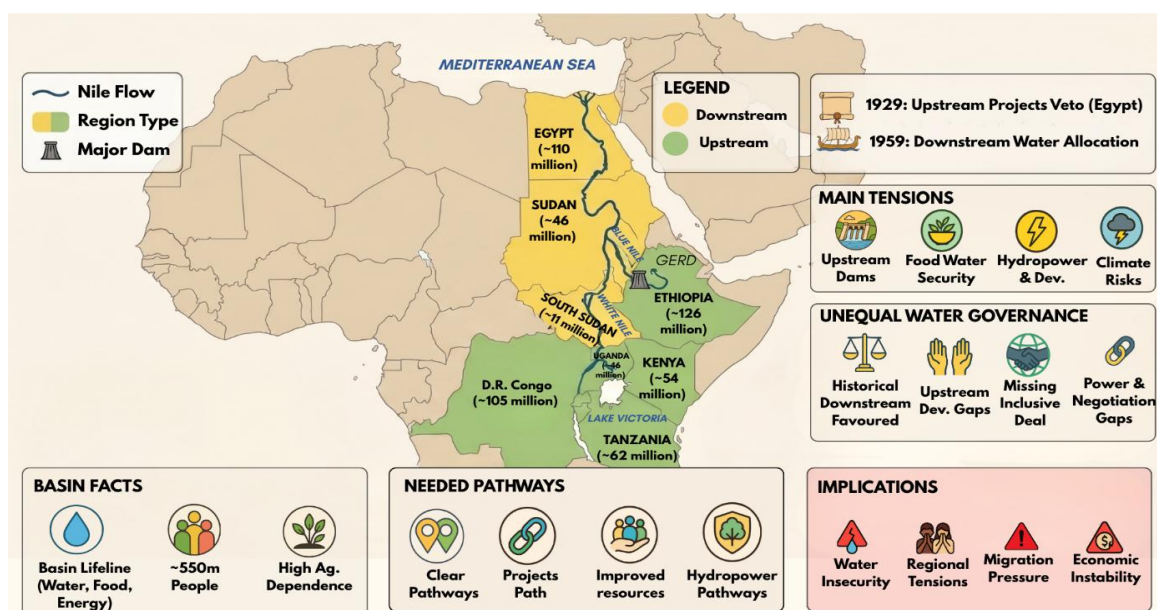


Figure 5. Nile Basin tensions and unequal water governance

Egypt has indicated that if the GERD reduces the water available for Egypt, that will be treated as a *casus belli*. This is a dangerous idea. It is not related by Egypt to any idea of reuse and recycling

of water, of the need for a small population, of the need to conserve water, of the injustice of the 1929 Note and the 1959 Treaty. Nor is there any idea of controlling population numbers or of conservation of water in Sudan. Equally disturbing is the fact that all these riparian countries are unstable, more or less, engaged in warfare to some extent (including combating anti-government forces), and not developing adequately. Libya is controlled by two rival governments and is a centre for migration to the rich north. Sudan is split between two military forces. South Sudan is in almost the same situation.

The enormous size of Egypt's population related to its resource base, including income from tourism, cannot be ignored. There is extensive poverty in Cairo, in Alexandria and elsewhere. Virtual or actual poverty is present also in Sudan and South Sudan. *Poverty is a harder fact than virtual water.* The injustice of relying in 2026 on the 1929 Note and the 1959 treaty is that in 1929, industrial needs and population growth were relatively insignificant in Egypt and Sudan then; and slight or non-existent in the other countries. For Egypt and Sudan to hold a majority allocation of water in 2026 is asking for trouble. Every human being has a right to justice.

Egypt and Sudan and South Sudan should all focus on water conservation and reducing their populations by family planning programs, with additional programs for development in line with the Sustainable Development Goals. This can also be said about all the other riparian states whose use of the Nile is crucial to their lives [22-24].

And can it not be said also to Turkey, which controls by many dams the waters flowing to the Tigris and Euphrates which are the source of life for Iraq? And to Iran which controls rivers flowing into Iraq? And what of Iran's current predicament in regard to the water supply of Tehran, from which its government has said the population - a mere ten million human beings - should be evacuated, because the water is running out? And what of Iran's actual status as a country 5 times larger than France, with 85% of its territory desert, a perpetual problem of sandstorms, and the almost entire loss of Lake Urmia [25]?

The most extreme form of these contrasting issues may be that of Palestine and Israel. Israel effectively controls all the water of Palestine. This cannot be justified. As for Israel, for several decades. Israel promoted economic use of water and sustainable agriculture by offering learning visits to appropriate researchers and practitioners in developing countries. Israel has not, however, used these measures by active diplomacy towards its regional neighbours. MENA includes Israel. Israel has done little to ingratiate itself with its Arab neighbours. Yet promoting effective use of limited water by visits from or demonstrations in MENA could promote acceptance of Israel. Although Israel has developed economic forms of irrigation, these have not been used to bring

peace to the region. Palestinians see their need to use their water *as being also their right to that water*.

Perhaps matters can be improved on the simple basis that the existing solutions can be applied, whatever their source, because the information is more or less in the public domain. Within Morocco, the company Agromillora, at Benslimane, produces many sapling trees each year by advanced propagation techniques. These can be replicated in other parts of MENA, with the same good effect. Covered areas of lightly watered plants in a controlled environment can produce food crops and plants for producing ground cover and re-establishing forest, where intercropping can be practised.

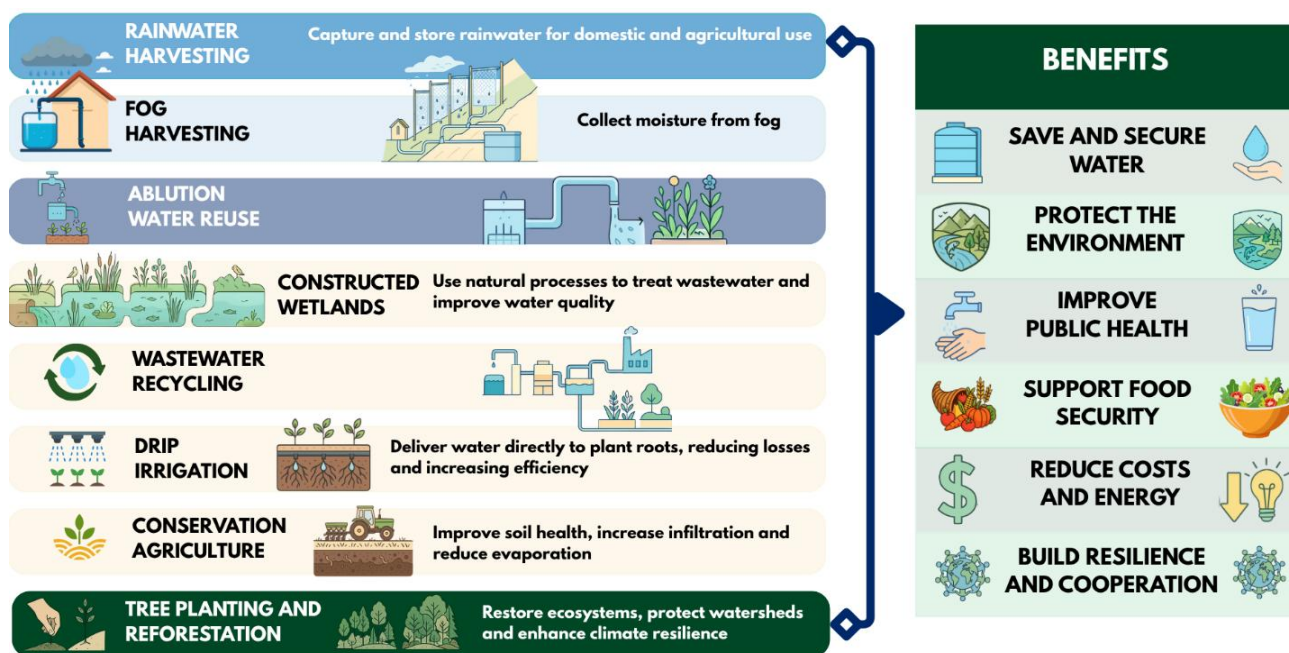


Figure 6. Adaptive water solutions for MENA

Drip or trickle irrigation instead of sprinkler irrigation can conserve water and produce adequate quality.

Discarding tillage and applying Conservation Agriculture avoids losing topsoil, which is a major problem, as it seems to justify amounts of chemical pesticides and chemical fertilizers that cause financial burdens and affect human health. Using compost and encouraging its production for export to other countries is desirable. Morocco has impressive fishing and poultry industries. The debris generated can be used as components of compost for sale throughout MENA [26].

Another question about water is its contamination. Britain has had numerous problems of discharge of raw sewage to its rivers and into the sea, after the businesses which were responsible for water supply failed to maintain the systems but paid profitable dividends to their shareholders. Consumerism is never a good idea. Margaret Thatcher's idea of selling off British water resources to private companies was self-destructive. The pollution of drinking water on land is accompanied by pollution of the world's ocean - in every country.

For all the years since the Stockholm Conference on the Environment, in 1972, mankind has pretended that 'the environment' is some kind of problem which can be managed and which does not require any serious change of direction in human affairs. All the evidence is against this idea [27]. The data may be denied, but they cannot be obliterated. The political system everywhere is ill adapted to these facts and in some cases, notably the United States, it actively pretends that the facts are 'a hoax'.

Just as risks which were once calculated according to probability have become more difficult to calculate, because the least probable has become more normal than abnormal, so we may now have to consider remedies to problems by innovatively using existing devices but for extraordinary purposes in a world where they have become more normal than extraordinary. For example, desalination is extensively used by Kuwait. Perhaps oil tankers should be repurposed to transport freshwater from countries with a surplus (e.g., Ireland, France) to countries with a deficit (e.g., MENA). Further, stormwater including monsoon floodwaters, could be salvaged and transported from the Indian subcontinent to MENA. Harvesting rainwater is normal in India and Australia. Why should not stormwater be harvested? Why should not fog water be harvested [28]?

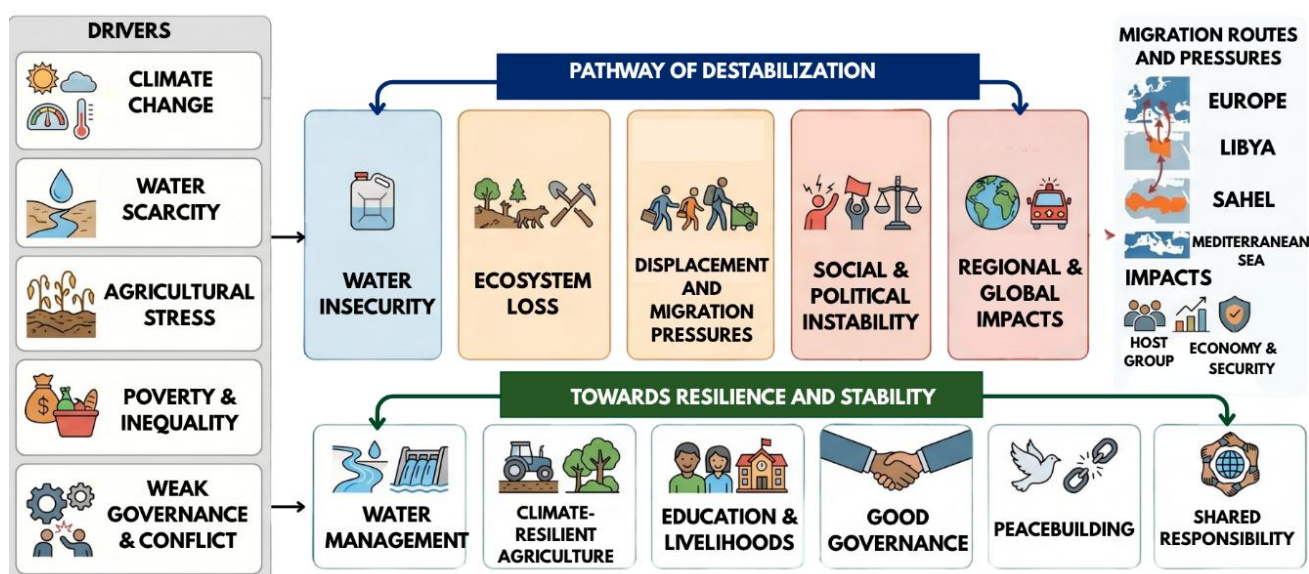


Figure 7. Water insecurity, migration, and ecological destabilization

At the end of 2025, Greece faced a fivefold increase in migrant arrivals via the Libya route [29]. From January to late December, 19,948 reached Greece from Libya. In all 2024, there were only 4,935. This increase of *more than 400 percent* does not include the many who must be presumed to have drowned in the frequent failures of vessels carrying people to Greece from Libya. On December 27th 2025, a commercial ship picked up 109 migrants south of Gavdos (an islet to the south of Crete) and brought them to Paleochora (on Crete itself). Of these 49 were Egyptian nationals; 40 were Bangladeshis; 20 were from Sudan. In a major rescue in early December, most of the 540 people on board a fishing vessel south of Gavdos were Bangladeshi. This is a devastating burden on resources. Misery is being encouraged by false hope for a better life. Yet the migrants who continue to make these terrifying journeys could stay at home and prosper with different national policies in their countries. World politics must confront the world problematic. National politics must confront the world problematic. Boumedienne was right, but the north and the south must remain in the same world, changed by better policies. Remember one thing. The environment does not need us. We need the environment [30-32].

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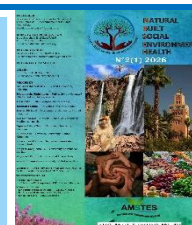
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Water Flow in the Tigris-Euphrates Basin: Challenges to Long-Term Solutions Under State Sovereignty, Political Instability, And Climate Change Uncertainties

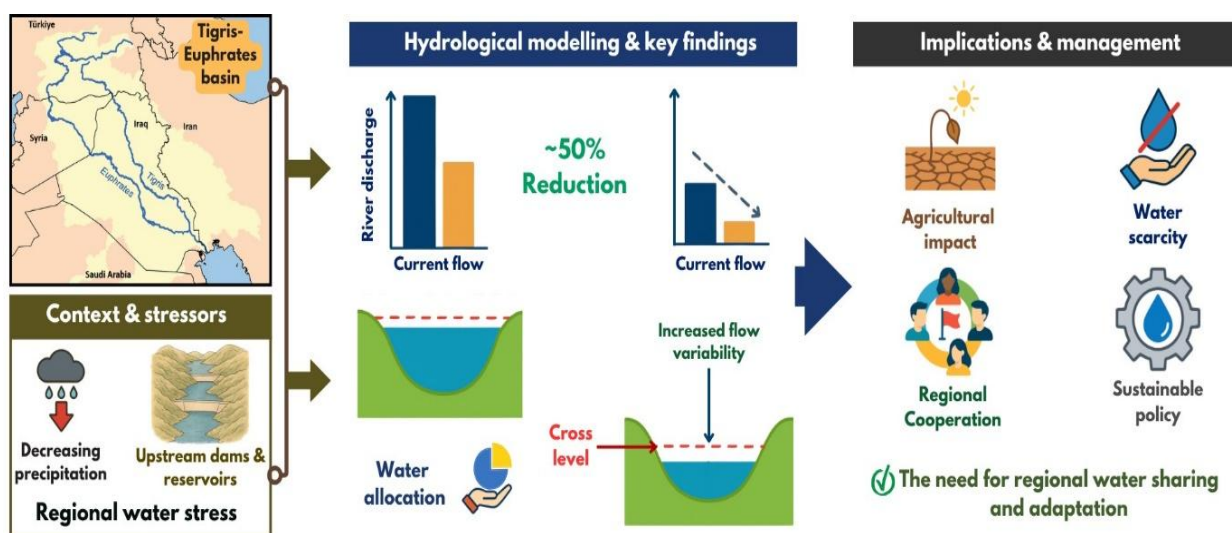
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Graphical Abstract

The Tigris and Euphrates are the Middle East longest permanent rivers, shared among Türkiye, Iraq, and Syria. Challenges to the natural flow of these waters include state sovereignty amid international anarchy, political instability, and climate change. Recognizing the historical rights of each state and focusing on temporary bilateral agreements among these states is an appropriate approach to sharing the water.



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Abstract: The Tigris and Euphrates rivers and their tributaries are the lifeline of Iraq and parts of Syria, and they are significant water resources in Türkiye and Iran. Iraq is known in historical texts as the land between two rivers, or Mesopotamia, which illustrates the importance of the rivers to this country. Recent hydraulic structures in upstream states, along with climate change factors, have caused interruptions to the flow of the rivers and reduced their waters, thereby creating a huge water deficit in downstream Iraq. This paper reviews the profound significance of the rivers for Iraq. It argues that state sovereignty, political instability, and increasing fluctuations in precipitation because of climate change pose considerable challenges to the natural flow of the rivers, making short-term bilateral solutions more practical.

Keywords: Tigris, Euphrates, Uncertainties, Temporary solution, Iraq.

Introduction

The Tigris and Euphrates are the longest permanent rivers, with their entire catchment basins located in the Middle East. The rivers and their tributaries are shared by Türkiye, Iran, Iraq, and Syria. They flow mainly in southeastern Türkiye: the Tigris enters Iraq from the north and is joined by several important tributaries coming from Türkiye and Iran, and the Euphrates flows through the eastern areas of Syria before entering Iraq. The two rivers get close to each other near Baghdad and continue through the lowlands in southern Iraq where they merge, forming the Shatt al-Arab River, which flows for about 200 kilometres before it empties into the Persian Gulf. The Tigris and Euphrates, along with their tributaries, are significant water sources in Türkiye's southeastern and Iran's northwestern areas, and they are the lifeline for Iraq. Syria's eastern areas also rely heavily on the Euphrates' waters. Ancient Iraq was called Mesopotamia, which means 'the land between two rivers', or referred to as the 'black land', indicating the fertility of its soil for agriculture. It was also known as 'The Fertile Crescent'. It is safe to say that life and human civilization would not have been possible in most parts of Iraq without the Tigris and Euphrates.

The natural flow of the Tigris and Euphrates rivers faces problems from three sources: the power and rights exercised under state sovereignty; conflicts between the riparian states; and climate change, which causes significant changes in precipitation patterns. State sovereignty amid international anarchy means, in the context of this paper, that Türkiye and Iran claim ownership over the waters and feel unrestricted in how they use the waters that flow in their territories, and how much water they store or release to downstream states. Türkiye and Iran are unrestrained by international treaties on transboundary watercourses, and international anarchy is often used by states to bypass their international obligations. Political instability entails tensions and conflicts between the rivers' riparian states that impact their inter-dynamics and relations. The four riparian states covered in this paper have had volatile and troubling relationships in the past decades; for example, Türkiye was once on the verge of invading Syria over the latter's refusal to hand over the leader of a rebel group. Climate change means that rainfall and snowfall, which constitute the sources of the Tigris and Euphrates, are more unpredictable than before; there is generally less rain and snow, and the Middle East is getting drier. This reduction of rain and snow can be used by upstream states to justify their water policies.

Following the introduction, there are four other parts and the conclusion. Part II reviews the transboundary Tigris and Euphrates rivers and their importance to the riparian states, focusing on Iraq since it is the most vulnerable state to changes in the water flows in the basin. Part III studies the issue of state sovereignty under international anarchy and what it means for controlling the

waters. Part IV surveys political tensions and conflicts between the riparian states. Part V reviews the impacts of climate change on the riparian states. The conclusion suggests that these factors make short-term bilateral solutions more practical than long-term multilateral solutions for the flow of the rivers. The main question this paper attempts to answer is: what challenges do state sovereignty, political instability, and climate change uncertainties pose to the flow of the Tigris and Euphrates rivers?

Part II: The Tigris and Euphrates basins: Türkiye, Iran, Iraq and Syria

Cradled entirely within the Middle East, the Tigris and Euphrates hold the distinction of being the region's longest permanent rivers: Türkiye, Iran, Iraq, and Syria. The rivers served as vital conduits for the development and sustenance of ancient civilizations in the Mesopotamian plain, in modern Iraq, such as Sumerian, Ubaid, Akkadian, Babylonian, Seleucid, Sasanian, and, later, Islamic and Ottoman. Irrigated agriculture and a system of natural and artificial water canals facilitated food production, mainly grains, and transportation, which stimulated invention, innovation, and development in many other sectors. The rivers are extremely vital for Iraq. They provide freshwater for domestic use, agriculture, industry, and recreational purposes. Iraq was rich in water resources from the two rivers until a few decades ago when Türkiye, as well as Iran and Syria, began building dams on the sources of the rivers. Iraq experiences a huge water shortage almost every year and is close to being characterized as a country with acute water scarcity [1,2].



Figure 1. The Tigris and Euphrates rivers and their catchment areas
<https://archeorient.hypotheses.org/36681>

The Tigris and Euphrates headquarters are located in the Taurus Mountains in southeastern Türkiye, where a considerable amount of rain and snow falls annually. The rivers take separate paths toward the south of the country; the Tigris enters Iraq at the town of Fiesh Khabur, and the Euphrates enters Syria at the city of Jarablus, then crosses the Iraqi border at the town of al-Qaim. The rivers get close to each other in the vicinity of Baghdad but remain separate and flow through central Iraq. They finally converge near the city of Al-Qurnah, where they form the Shatt al-Arab, which eventually flows into the Persian Gulf.

The Tigris waters increase in Iraq because of significant tributaries that originate in southwest Iran and the Kurdistan Region of Iraq. The Euphrates only loses water in Iraq, as it is not joined by any

tributaries. The Tigris runs for about 1,800 km and the Euphrates for 2,780 km to 3,000 km; the two rivers' basins cover an area of about 917,103 square km, located in Iraq, Türkiye, Iran, Syria, and Saudi Arabia. The estimated annual discharge of the Tigris is about 52 billion cubic metres (BCM), and the Euphrates is 32 BCM, with Türkiye being the main contributor to their waters among the riparian countries, which also include Iran, Iraq, and Syria. Both rivers' annual flow fluctuates significantly from one year to another and from one season to another; it is usually high in March and April and low in September and October. Generally, since the construction of hydraulic structures in upstream states combined with the impacts of climate change, the rivers' flow has drastically decreased [3].

The Tigris River originates in the highlands of Hazar Lake in Türkiye, surrounded by the Taurus Mountain chain. Its length inside Türkiye is approximately 400 km before it reaches the Iraq-Syria border. The river is shared between Türkiye and Iraq; but, its catchment basin includes Iran and Syria as well. Türkiye contributes 51% of the Tigris River's annual water volume through its water resources, which include three main tributaries: Batman, Garzan, and Botan, as well as the Greater Zab River (also called Upper Zab), which first flows into Iraq, where it receives water from other tributaries before it joins the Tigris. The volume of water generated for the Tigris within Türkiye is between 18 and 21 BCM. About 21% of the length of the Tigris lies in Türkiye; the rest is in Iraq, approximately 1,418 km. Besides the Greater Zab, three other tributaries join the Tigris inside Iraq: Khabur, Lesser Zab (also known as Lower Zab), and Diyala (known as Sirwan in the Kurdish areas of Iraq), which add another 25 to 29 BCM to the river [4,5].

The Euphrates River originates near Mount Ararat, close to Lake Van, and is joined by two significant tributaries: the Murat-Su and the Kara-Su (or Frat-Su), which, in effect, constitute the majority of the river's water. The volume of water generated for the Euphrates within Türkiye is between 31 and 32 BCM. The river runs through Türkiye for approximately 1,230 km. Within Syria, the Euphrates receives a small amount of water from two tributaries: the Balikh and the Khabur rivers. Syria contributes about 10% to the Euphrates River, which flows through the eastern part of the country for approximately 710 km. The river's length in Iraq, where it receives no tributaries, is approximately 1,060 km. The catchment basin of the Euphrates includes Saudi Arabia as well. The total annual discharge of both the Tigris and Euphrates is approximately 84 BCM. This amount fluctuates greatly depending on the needs of upstream states, especially since the dams can hold a significant amount of water, as well as annual precipitation [2,6].

There are many dams built upstream in Türkiye on the Tigris and Euphrates rivers as part of the ambitious Southeastern Anatolia Project, known by its Turkish acronym, GAP. On the Tigris and

its tributaries, there are Ilisu Dam, which is the largest with a storage capacity of 10.4 BCM; Dicle Dam; Kralkızı Dam; Cizre Dam; Batman Dam; Alkumru Dam; Silvan Dam; Garzan Dam; Çetin Dam; Devegeçidi Dam; Silopi Dam; Göksu Dam; and Hacıhıdır Dam. On the Euphrates and its tributaries, there are Atatürk Dam, which is the largest with a storage capacity of 48.7 BCM; Keban Dam; Karakaya Dam; Birecik Dam; Karkamış Dam; Bağıştaş I & II Dams; Sırımtaş Dam; Hancağız; Kayacık; and Çamgazi Dams. The Euphrates River flow is also retained at several dams in Syria before it enters Iraq. The largest and most significant is the Tabqa Dam, with a storage capacity of 11.7 to 14.1 BCM. There are also Tishrin Dam; Baath Dam (also known as the Mansoura Dam); Halabiye Dam; and Khabur River tributary [7]. These dams have a great impact on Iraq as a downstream state. They constitute a real threat to the flow of the rivers to Iraq. There are many dams on the Tigris and Euphrates rivers and their tributaries in Iraq too; but, they are not significant in the sense of constituting a threat to another downstream state since Iraq is the last downstream state on the rivers.

There are dams inside Iran built on the main tributaries that flow to Iraq and join the Tigris River. On the Lesser Zab (also called Little Zab and Lower Zab), there is Zardasht Dam, which is the largest and has a storage capacity of 380 to 387 million cubic metres (MCM); Silveh Dam; and Kani Sib Dam (also called Kani Siv or Kani Sew). There are many dams and tunnels on the Sirwan River in Iran as part of the ambitious Tropical Water Project (TWP). Daryan Dam, which is the largest with a storage capacity of 316.3 MCM, feeds the Nowsud Water Conveyance Tunnel, which transfers water to other parts of Iran. There are also Garan Dam; Gawshan Dam; Azad Dam; Zhavveh Dam; Herwi Dam; and several other smaller dams [8-12]. Dams in the upstream states are a curse for Iraq; they deny Iraq its due share of the waters, but they can also be a blessing if upstream states cooperate with Iraq.

Iraq is heavily reliant on the waters of the Tigris and Euphrates rivers, which provide 90% to 98% of the country's surface water. The rivers and their tributaries are the primary sources for drinking water, domestic use, irrigation, industrial and energy use, and recreational activities in a country that is largely arid (except for its north and northeast), also known as the Kurdistan Region of Iraq, and rain is scarce in its western and southern parts. Iraq's rapidly growing population, which is now close to 50 million, is increasingly vulnerable to any changes that occur in the flow of the waters. All the riparian states need to understand this difficult situation and work together, bilaterally, as we argue, to help mitigate the impact of climate change and ensure that the waters do not face continuing disruption to downstream states [13].

Part III: State sovereignty

State sovereignty is problematic for the natural flow of transboundary rivers. The modern nation-state exercises power over a defined territory and population and rejects outside interference. The sovereign rights inherited from the Peace of Westphalia in 1648 and enshrined in the United Nations Charter in 1945 allow internationally recognized modern nation-states to hold exclusive power within their defined borders, including the management of the natural resources, such as water. The world is divided along borders, creating nearly 200 nation-states, big and small, with their own separate governments, distinctive jurisdictions, and political institutions. There are hierarchies and order within a state. Outside a state, there is no such thing; there is anarchy [14-16].

International anarchy refers to a system in which the nation-state is the highest authority to make decisions and enforce laws within its borders. There is no global central sovereign authority that can impose laws on nation-states or make decisions for them. The sovereign nations see themselves as equal in terms of having legitimate power within their territories, viewing no authority above themselves and negotiating on equal footing when trying to resolve disputes that occur between them. This situation emboldens upstream states to behave independently on the international watercourses [17].

The problem is that, similar to individuals, states are self-interested and that survival and dominance are essentially the driving forces behind their behaviour in international relations. States seek survival when situations are difficult for them. A state's priority is to endure the difficult phase, no matter how long. Then, when things get easier and the pressure is reduced, states seek dominance and control; they attempt to dominate and overpower other states and influence their affairs if they can. This is human nature according to realism, which is the mainstream school of International Relations (IR), and it is reflected in states' behaviour, which exhibits the human survival instinct. Both survival and dominance tendencies work against the natural flows of international watercourses [18].

During the survival period, and because of the rising demand for water, drought, etc., the upstream state may prioritize its own needs and use the water for irrigation, domestic use, and various other activities. During the dominance period, the upstream state uses the water as leverage against the downstream state to obtain concessions and make it respond to its demands, which can be political, economic, or of any other type. These behaviours are made possible by sovereign rights exercised in the absence of a global central authority and a lack of any global institutions with the power to check states' behaviours and hold them accountable for their actions. Morality, ethics, and the

Christian principle 'love your neighbour as yourself' are not enough to prevent a state from harming another state [19,20].

Power plays an important role in international relations. Military capabilities, whether a state possesses them or allies itself with states that possess them, can strengthen a state's position regarding the flow of international rivers and other issues related to its foreign policy and international relations. Downstream states can use their military power to threaten upstream states to release water or not block the flow of water in the first place. Economic and diplomatic power can similarly bolster the position of a state. For a long time, Egypt prevented Ethiopia and other upstream states from building dams and water projects on the Nile River, receiving the lion's share of the Nile waters. But power changes, and so do the abilities of a state to secure its waters flowing in its territories or coming from foreign territories. Egypt could not stop Ethiopia from building the Grand Ethiopian Renaissance Dam after years of success in retaining the majority of the Nile waters for itself. Power can be more dangerous when it is in the hands of upstream states, as they are already in a stronger position because of the location of the river's sources [21].

Sovereignty under international anarchy allows states to evade their international obligations. States tend not to fear the consequences of their actions, as the global institutions that impose international laws and obligations either do not exist or are weak and unwilling or unable to impose sanctions or punish the breaching states. States that are not signatories to international treaties can more easily evade their responsibilities. Türkiye and Iran, both upstream states to Iraq, are not signatories of the 1997 United Nations Convention on the Law of the Non-Navigational Uses of International Watercourses; hence, they take no responsibility to follow the provisions of the treaty. Moreover, both Türkiye and Iran are militarily and economically stronger than Iraq. This puts Iraq in an unfavourable position concerning its share of the Tigris and Euphrates waters [22-24].

The contemporary international political system does not help downstream states secure a fair share of water from international watercourses. The system, which consists of independent, sovereign nation-states, allows upstream states to treat downstream states unfairly and leaves downstream states with few options to rectify the injustice. Injustice and unfair treatment by some states against others will continue as long as anarchy exists. According to the school of realism, international anarchy is a permanent feature of international relations [25,26].

Part IV: Political instability

Instability is a defining characteristic of interstate relations in the Middle East. The Middle Eastern states do not have stable relations with one another because of historical rivalries, conflicting interests, ideological differences, and their competition for approval and favour by powerful states

outside their region. The countries' rulers lack popular legitimacy and usually rely on outside powers for protection. Moreover, outside powers' economic interests in oil and gas, as well as their geopolitical interests, often work against political stability. In fact, the very instability is beneficial for them in the sense that it makes their presence necessary and leads to profitable arms deals, security, and other agreements [27].

The riparian states of the Tigris and Euphrates rivers have had conflicts and rivalries in the recent past, with implications for their wider relations, including cooperation over the shared waterways. The four riparian states have also experienced periods of good relations; examples include the rapid improvement of relations between Iran and Iraq following the overthrow of the Sunni-oriented Iraqi regime under Saddam Hussein in 2003 and the formation of a Shia-majority government in Iraq, which aligned with Iran's state ideology. Another example is the rise to power of a Sunni and pro-Turkish militia group in Syria in 2024, which improved relations with Türkiye. Normally, these changes would not lead to long-term institutional relations or provide enduring stability. The general trend of relations between these countries is instability [28].

Türkiye and Syria, the former being upstream on the Euphrates River relative to the latter, have experienced years of bad relations in the twentieth and twenty-first centuries. Syria has been a major security concern for Türkiye. This became more apparent following the 2011 uprising in Syria, for which Türkiye showed its greatest support. Türkiye backed the opposition forces, became a safe haven for the opposition groups, trained them, funded them, and declared that the Syrian regime must go. Prior to the uprising, the two states had major disagreements regarding border issues, water rights, and divergent foreign policies. Following the uprising and the formation of Kurdish groups linked to armed opposition groups, Türkiye intervened militarily in northern Syria and controlled a long strip across the border. It is difficult to say that both states can cooperate long-term, knowing that the present Türkiye-Syria relations are largely person-to-person and party-to-party. If the Turkish ruling party, the Justice and Development Party, loses power, or President Recep Tayyip Erdoğan is gone, or if power changes hands in Syria, the recent improvement in their relations may vanish quickly [29-31].

On the Euphrates line and regarding Syria and Iraq: their relations have been characterized by bitter and enduring conflicts since the seizure of power by the rival branches of the Baath Party in both countries in 1963. The leaders of the ruling Baath Party, Hafez al-Assad in Syria and Saddam Hussein in Iraq, had ideological differences and personal power struggles, competing with each other for domination in affairs concerning the Arab nation. There were several assassinations attempts against the Baath leaders in both countries, which often led to accusations and threats of

retaliation. Syria supported Iran in its eight-year war against Iraq from 1980 to 1988, creating problems for Iraq's oil exportation and the flow of Euphrates waters to Iraq. Iraq backed Syrian opposition groups and supported coup d'état attempts in Syria. Relations slightly improved following the fall of Saddam Hussein in 2003 and the rise of Shia Muslims to power in Iraq. The Assad family in Syria were Alawites, which is a minority Shia group. But, the Kurdish political movements and security became serious issues between the two states. The fall of Bashar al-Assad in 2024 and the rise to power of Ahmed al-Sharaa, formerly known as Abu Mohammad al-Julani, has initiated another tense phase in Syrian-Iraqi relations. Al-Sharaa is recognized by Iraq as a terrorist, and there are serious charges pending against him. New ideological differences, as well as political, security, and economic issues, all exist with no horizon for resolution soon [32,33].

Türkiye and Iraq's relations, as the two riparian states of the Tigris River, have been partially cooperative and largely contentious, with major issues centred on land disputes. This is embodied in Türkiye arguing that it should have sovereign rights over the entire Mosul Wilayat, which constitutes about one-third of total Iraq's land, as well as on security and water resources. For many years, Iraq's Kurdistan Region mountains in the northeast of the country have been home to Kurdish guerrilla groups fighting the Turkish state over the political rights of the Kurds in Türkiye. Türkiye has supported the Iraqi Kurds on many occasions and in many respects, which has in effect weakened the political and economic powers of the Iraqi central government. The lack of cooperation was also the result of Türkiye's political orientation for most of its contemporary history. The modern Turkish state, officially known as the Republic of Turkey, founded in 1923 (the country's name changed to Türkiye in 2022), under the legendary leader Mustafa Kemal Atatürk and other subsequent governments until the coming to power of the Justice and Development Party (known by its Turkish acronym AKP or Ak Parti) in 2002, was more Western-looking and largely ignored its ties with the Muslim world. This has negatively impacted its relations with Muslim countries, including Iraq. Despite the change in attitude and foreign policy of Türkiye under AKP, Iraq's political and security upheavals since 2003 have led this country to be viewed by Türkiye in a threat-centric perspective. Both countries are far from long-term agreements on the flow of the shared watercourses [34-37].

Relations between Iran and Iraq, as riparian states of the two important tributaries of the Tigris River, have fluctuated greatly over several decades between cooperation and alliance, as well as rivalry, dispute, and war. Both states had major disagreements over the demarcation line between them in the Shatt al-Arab waters; both have had a tendency for regional dominance, along with differing ideologies and political orientations. The peak of their conflict was their eight-year-long war, 1980-1988, which is recognized as the longest conventional war of the twentieth century. It

occurred following the Islamic Revolution in Iran in 1979, when the political and regional goals and ambitions of Iran's Ayatollah Khomeini and Iraq's Saddam Hussein clashed. Iraq saw the new regime as a direct threat to itself and its ability to manoeuvre in the Shatt al-Arab waterway, and Hussein believed that the Iranian regime would soon collapse if attacked. The war ended with the death and injury of approximately one million people from both sides, widespread devastation, and economic ruin. The relationship between the two countries has improved significantly since the collapse of Saddam Hussein's regime in 2003. Iraq's many internal security problems and political instability, international sanctions on Iran, and the United States' attempt to isolate Iraq from Iranian influence, along with Iran's sharply rising demand for water, have all meant that both states cannot reach long-term agreements over their shared rivers [38-40].

Part V: Climate change factors

The Middle East is under severe impact from climate change. The average temperature in the region is rising twice the global average, with huge implications for human life and the environment. In 2024, the Middle East experienced the hottest recorded year in its history; temperatures exceeded 50 degrees for many days in several countries, including Iraq. Dust storms are rampant and increasing, especially during the hot seasons, and desertification is expanding across the region. Water is under stress. Reports suggest a reduction of 50% in water per capita in the Gulf Region by 2050. This is disastrous since the region is already water-stressed. Agriculture is in decline as well, as it relies on water. The region increasingly relies on imports for its food needs. Immigration to urban areas and pressure on public health are other areas where climate change makes an appearance [41-43].

Türkiye is believed to be less affected by climate change because of its geographical location. This is far from the truth. Rising temperatures and a decline in annual precipitation are increasingly altering the country's hydrology. It impacts the headwaters of the Tigris and Euphrates. There is a decline in annual rain and snow in eastern Anatolia, and early melting of snow is increasingly noticeable. Snow, as a significant source of both surface and ground water in the eastern and southeastern highlands of Türkiye, is projected to decrease (55%-87%), and surface runoff is expected to decrease (26-57%) by the end of the twenty-first century. This threatens water security in Türkiye. In other words, climate change creates greater uncertainty regarding the availability of freshwater, making long-term agreements a less likely option [44].

Syria suffers from a severe impact of climate change. The most visible impacts are both the rise in temperature and drought, which are intense and devastating for the country. Subsequent years of drought in the past decades have hit the country hard, with devastating impacts on crops, livestock,

agriculture, and rural life, badly affecting over 1.5 million people. The country, and its northeastern region where the Euphrates flows through, suffers from an increasing decline in precipitation, plunging crop production, mainly wheat, and making the country more and more reliant on food imports. Climate change is the driver of desertification and land degradation in Syria. It has led to a rise in diseases and the number of people needing medical care, along with other social problems. The availability of freshwater is a growing problem in many parts of Syria, and the Euphrates is becoming more intermittent, which means that the country lacks reliable and renewable freshwater resources, which, in effect, reduces the chance of a long-term agreement with downstream Iraq [45,46].

Climate change is making a visible presence in Iran. It manifests in rising average temperatures, increased heatwaves, and as a result, an increase in heat-related illnesses, a decline in precipitation (the rainfall in 2024-2025 was just above 100 mm; the standard is 260 mm), a growing freshwater deficit in many areas, including the capital Tehran, the drying up of lakes and reservoirs, agricultural decline, loss of biodiversity, land and environmental degradation, etc. Iran recently witnessed five successive years of drought from 2020 to 2025, which put huge strains on the rapidly declining available freshwater, pushing the Iranian president to suggest that the capital city must be relocated from Tehran, a city of 10 million people, to the south of the country and on the shores of the Persian Gulf, where water can be obtained from desalination. Iran has many dams and tunnels on the tributaries of the Tigris River to collect water and then transfer it to other areas of the country where water is urgently needed for agriculture, regardless of the needs of downstream Iraq [47-54].

Iraq is experiencing the full range of climate change effects. It is among the countries which are most vulnerable to the impact of climate change. The rise in its average temperature is higher than the global average, exceeding 53 degrees in central and southern areas on some days in summer; loss of billions of cubic metres of water to evaporation; a decline in annual rainfall, except for some years such as 2025-2026; the drying up of the marshes in the middle and south, which means the loss of the habitat of water buffalo, fishery, and loss of biodiversity for millions of species, etc.; a decline in crop and fruit production such as oranges and dates; the expansion of desert lands swallowing arable land by 100 square kilometres every year; deforestation; increasing dust and sandstorms; and pressure on public services owing to immigration from rural areas to urban centres, etc. Iraq, as a downstream country desperate for water, is undoubtedly in favour of a permanent and stable flow of the Tigris and Euphrates waters - an ambition that is unattainable for reasons mentioned above [55-58].

Despite the findings of this study, two limitations must be acknowledged. First, primary materials in the form of interviews and data collected from direct field trips were not possible under the current circumstances in Iraq. Second, regarding the political aspect of the problem, apart from realism as a school of thought, other IR theories can be important for understanding the situation and also finding solutions. Nevertheless, this focused approach allows for a deep understanding of the nature of the problem. Future studies should consider using primary materials and examining the issue with other perspectives.

Conclusion

There has never been a war between Türkiye and Syria over the Euphrates waters, Syria and Iraq over the Euphrates waters, Türkiye and Iraq over the Tigris waters, or between Iran and Iraq over the tributaries of the Tigris waters; but there also has never been long-term cooperation and agreements over the flow of these rivers between these states. All riparian states of the Tigris and Euphrates have growing needs for water, but the sources that feed the rivers are under the increasing effects of climate change. Average annual precipitation is generally declining in all the Tigris and Euphrates basin countries.

Climate change makes grand and enduring cooperation between these states difficult, as it creates huge fluctuations in the annual flow of rivers and uncertainties for the future allotment of water to the states. The unlimited power that modern states exercise under the guise of sovereignty and the freedom they have over the management of their internal affairs, including the international watercourses flowing within their territories, along with the political tensions between them, creates problems for long-standing agreements and cooperation between the Tigris and Euphrates riparian states.

States in the Tigris-Euphrates basin have different preferences and abilities, and they feel the imperatives of climate change differently; hence, they have varying approaches to the shared waters. The historical rights of downstream Iraq and Syria, along with their desperate need for water, cannot be disregarded. On the other hand, upstream Türkiye and Iran do not publicly deny the rights of downstream states to water. Nevertheless, ideological differences, political orientations, and a bitter history of conflict and rivalries make these states unwilling to enter into long-term and multilateral agreements. Therefore, we propose coordination and short-term annual solutions.

Coordination is defined as ‘a process in which two sides learn to work with one another to forge commitments to cooperate with new rules of agreement.’ It is described as less regularized than cooperation, occurring in small and practical steps based on a common understanding.

Coordination is also less prone to politicization [59]. Despite political tensions and security threats, the four riparian states do trade and conduct business with each other on a large scale. They are interdependent on one another to a great extent for manufactured and agricultural products, as well as energy [60]. These countries are not going to abandon each other. Therefore, we suggest that Türkiye and Iraq over the Tigris River, Iran and Iraq over the tributaries of the Tigris, Türkiye and Syria over the Euphrates, and Syria and Iraq over the Euphrates can bilaterally coordinate with one another to develop short-term solutions regarding the flow of the waters.

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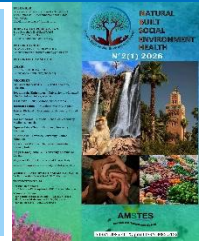
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Drying of the Panama Canal: Causes, Impacts and Mitigation Measures

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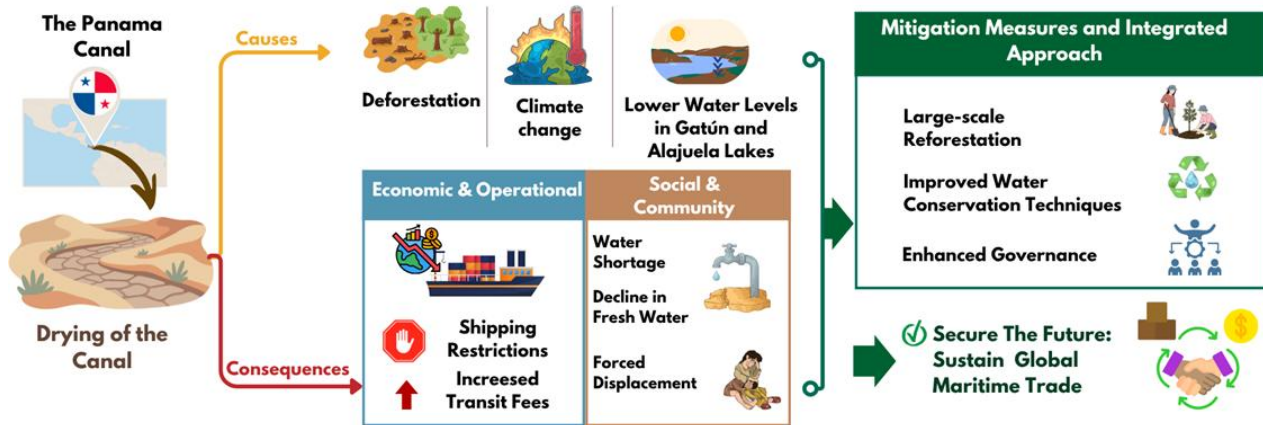
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Graphical Abstract

The Panama Canal is threatened by freshwater decline due to deforestation and climate change. Falling levels in Gatún Lake and Alajuela Lake disrupt operations and impact communities. Integrated solutions such as reforestation and improved water management are essential for its sustainability.



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Abstract: The Panama Canal is facing severe operational challenges because of declining freshwater availability. Mass felling of trees in the watershed area of the Canal has drastically reduced the region's ability to retain water and attract rainfall. Rising global temperatures and irregular precipitation patterns caused by climate change have disrupted the Canal's freshwater supply significantly. This has resulted in lower water levels in Gatún and Alajuela Lakes, freshwater sources for the operations of the Canal. These declining water levels have led to shipping restrictions, increased transit fees, and economic strain on Panama's economy. Local communities are suffering from water shortage, forced displacement, and socio-economic instability because of conservation projects and alternative water management strategies. This study examines the underlying causes, consequences, and mitigation measures related to the drying of the Canal. Some interventions are identified: large-scale reforestation, improved water conservation techniques, and enhanced governance to mitigate this crisis. The Canal requires an integrated approach to secure its future in sustaining global maritime trade.

Keywords: Panama Canal, Climate change, Gatún, Alajuela.

Introduction

The Panama Canal, a crucial waterway that facilitates global trade, has been experiencing severe challenges caused by declining freshwater availability since late 2022 [1]. As a lock-based canal, it relies on movement of immense volumes of freshwater from Gatún and Alajuela Lakes to operate its locks and enable ship passage [2]. Yet, increase in global temperatures and changing precipitation patterns have led to prolonged droughts thereby decreasing the replenishment rate of these reservoirs. In addition, extensive deforestation in the Panama Canal watershed area has exacerbated water shortage by reducing the capacity of the land to retain and regulate rainfall. A combination of climate change, mass deforestation, and mismanaged water conservation efforts has reduced water levels drastically to threaten the Canal's functionality. Without sufficient forest cover, rainwater runs off quickly, leading to lower infiltration rate and increased evaporation, depleting water sources further.

The Canal's water crisis has widespread economic and social ramifications. Reduced water level has led to transit restrictions, limiting the number and size of ships that can pass, thereby increasing congestion and shipping costs. Since the Canal handles nearly 6% of global maritime trade, such disruption impacts international supply chains, raising transportation costs, and delaying shipments worldwide. Additionally, local communities that depend on the Canal for economic activities face significant water shortage as available freshwater is diverted to sustain Canal operations [3]. The resulting scarcity has intensified socio-economic challenges, particularly for rural and indigenous populations, many of whom rely on agriculture and fisheries that are directly affected by diminished water availability.

Despite extensive research on climate change and deforestation globally, there appears to be no published study of their combined impact on the Canal's sustainability. Although several conservation efforts such as reforestation programs and advanced water management technologies have been proposed, their implementation has been slow and has often met with political and economic resistance [4]. As a result, the Canal authorities have proposed construction of new reservoirs and implementing water saving measures, but the feasibility and long term efficacy of these solutions require greater assessment. This study thus aims to analyse the primary causes of the drying of the Panama Canal, its implications on the economy and populace of Panama, and the effectiveness of the proposed mitigation strategies to ensure a sustainable Panama Canal. The purpose is to provide insights into sustainable solutions that balance environmental conservation with economic needs; offering a holistic understanding of the crisis for Panama in general and the world as a whole. The study will thus bridge the gap between environment and economic needs by providing a comprehensive analysis of the Panama Canal's water crisis, integrating climate,

environmental, economic, and social perspectives. Additionally, the study will assess the long term economic risks associated with water shortages, the impact on vulnerable communities and the practicality of proposed mitigation strategies for Panama. The findings will contribute to informed policymaking and future research on sustainable water resource management in major trade corridors worldwide.

The paper begins with a literature review of the various studies undertaken by scholars, environmental experts, and policy institutions on the Panama Canal's hydrological challenges, with a focus on climate-induced droughts, impact of deforestation on watershed dynamics, and existing mitigation frameworks including reforestation programs, reservoir expansion, and advanced water conservation technologies. This will be followed by an analysis of the causes of drying of the Panama Canal, the multifaceted impacts of reduced water levels on trade and local communities, and discussion of proposed and implemented measures to address the present water crisis.

Literature Review

Drying of the Panama Canal has become an area of concern because of severe economic, social, and environmental repercussions. Accordingly, the Canal has been an area of study for researchers since the late 2000s after climate variability, unsustainable land use, and declining freshwater availability began visibly to affect its operational efficiency and long-term sustainability. Hence, it is important to examine key studies that have investigated the individual and collective causes associated with drying of the Canal. The earliest of these studies was by Estoque *et al.* [5] that highlighted the water concern for the Panama Canal. This study showed the way for many other studies that focused on climate change and deforestation as the primary cause for water shortage. But, significant gaps remained in understanding the long term consequences of water shortage and effectiveness of proposed mitigation measures. Hatvani *et al.* [6] found that the region has witnessed a 30% decline in annual rainfall over the past two decades caused by increasing global temperatures resulting in reduction of freshwater in the Gatún and Alajuela Lakes. Similarly, Aguilar and Naranjo [7] found that intensification of the El Niño phenomenon has prolonged droughts thereby exacerbating water scarcity and forcing the Canal authorities to impose transit restrictions. Without adaptation strategies, such climate related challenges will continue to disrupt Canal operations.

Since mass deforestation in the Panama Canal watershed has significantly reduced the region's ability to retain water, Fotos *et al.* [8] conducted a study demonstrating how extensive tree cutting has led to increased soil erosion, decreasing groundwater recharge and accelerating surface runoff.

They observed that this phenomenon prevents effective rainfall absorption, causing rapid depletion of freshwater reserves. Olson and Tornoe [9] found that deforestation in the Gatún Lake watershed has reduced water retention by 15%, leading to increased evaporation rates and lower lake levels, suggesting that reforestation efforts could play a vital role in reversing these trends.

Others have studied the economic impact of declining water levels in the Panama Canal. Kumar [10] estimated that a 20% decrease in Canal traffic because of water restrictions led to loss of billions of dollars annually in global trade. As the Canal becomes less reliable, shipping companies tend to seek alternative routes, reducing the economic competitiveness of the Panama Canal. Extending the argument, Aguilar and Naranjo [7] note that prolonged water shortage could permanently lower Panama's GDP by up to 5% through reduced revenue from Canal operations and associated economic activities.

The resulting water crisis has led to severe social and environmental challenges. Thomas [11] reported that prioritizing freshwater for Canal operations has worsened water shortages in nearby communities, leading to conflicts over resource allocation. Many rural and indigenous populations depend on the Canal's watershed for drinking water, irrigation, and fisheries. Olson and Tornoe [9] examined the environmental impact, revealing that reduced water levels have disrupted aquatic ecosystems, leading to habitat degradation and biodiversity loss in regions surrounding the Canal.

Accordingly, a number of mitigation strategies have been proposed. Suman *et al.* [12] explored the feasibility of constructing additional reservoirs to supplement the Canal's freshwater supply. Their study noted that such projects would require significant infrastructure investments and might displace local communities. Sasidhar [13] investigated the potential of water saving locks and advanced recycling systems, estimating that these innovations could reduce the Canal's freshwater consumption by up to 40%. Loewenberg [14] emphasized the importance of reforestation, suggesting that restoring tree cover in the watershed could significantly improve water retention and prevent further environmental degradation.

Despite extensive climate change research in Panama since 2007 [15] and hence research on the Panama Canal's water crisis, several gaps remain. Many studies focus on climate change and deforestation in isolation, but few examine their combined impact on the Canal's sustainability. Although some research highlights immediate economic losses, there is limited analysis of how prolonged water shortage could affect global trade and Panama's economy in the long term. Most studies have not explored the long term consequences of water shortages on local communities, including displacement, food insecurity and socio-economic instability. There has not been extensive study of the various mitigation strategies proposed, their real world feasibility, cost-

effectiveness and potential trade-offs. This study will focus on underlying causes, consequences, and mitigation strategies related to the drying of the Canal.

Working and Layout of the Panama Canal

The Panama Canal is a lock-based artificial waterway that connects the Atlantic Ocean (via the Caribbean Sea) to the Pacific Ocean, cutting across the Isthmus of Panama. Spanning approximately 80 kilometres, the Canal allows ships to avoid the much longer and more hazardous route around the southern tip of South America via Cape Horn. The core of the Canal's operation is its system of three lock complexes, Gatún Lock complex on the Atlantic side, and the other two, Pedro Miguel and Miraflores Lock complexes on the Pacific side as seen in Figure 1. These gigantic locks raise ships up to the elevation of the Gatún Lake (about 26 metres above sea level) and then lower them back down as they exit on the Atlantic side. The system relies entirely on freshwater, mainly from Gatún Lake and Alajuela Lake, to fill the lock chambers, enabling the transit of vessels through changes in elevation for either side movement, viz., the Atlantic to the Pacific (eastbound or north-to-south transit) and the Pacific-to-Atlantic (westbound or south-to-north) following different lock sequences and timing. The Atlantic-to-Pacific uses three-step Gatún Locks to lift ships 85 feet, followed by single-step Pedro Miguel (31 feet down) and two-step Miraflores (54 feet down). The Pacific-to-Atlantic movement follows the reverse order.

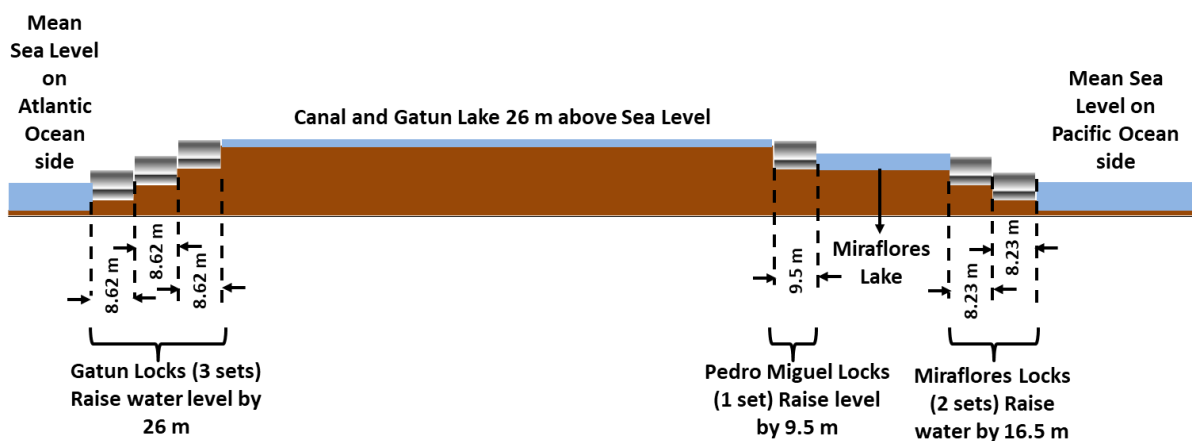


Figure 1. Layout of the Panama Canal (*Source: Authors*)

The flooding or draining of these Locks uses gravity-fed water from the reservoirs. To accommodate the growing size of ships, the capacity of the Canal was enhanced in 2016 by adding a third set of locks known as the Panama Canal Expansion (or “Neo-Panamax” Locks). This expansion allowed vessels of up to 366 metres in length to pass through. The freshwater-dependent design, though effective for nearly a century, makes the Canal vulnerable to shifts in regional

rainfall and water availability. A typical transit using Neo-Panamax Locks requires about 422 million litres of freshwater, of which nearly 40% is flushed into the sea after each passage whereas the older locks flush out 50% of the 200 million litres used [13]. For the Canal to function efficiently, Panama's wet season (May-December) must recharge reservoirs. But, water inflow has been reduced by 20-50% because of changes in El Niño and climate-driven events, increased evaporation and ship-induced salinity intrusion. Projections show that under high emissions the region is likely to experience droughts every 7-10 years by 2050 [16].

Causes of Drying of the Panama Canal

As discussed, the Panama Canal is a freshwater dependent, lock based canal. Each of the older lock chambers require about 98 million litres (26 million US gallons) (even after reusing 50% of existing water) of freshwater per fill. The new basin requires nearly 200 million litres (about 52.8 million gallons) (even after reusing 60% of existing water) to lift a vessel, drawn directly from Gatún Lake via gravity [13]. Owing to the surge in shipping and movement of larger vessels across the Canal, reducing water supply caused by drought, climate change, and deforestation, and an increasing population of Panama driven by a population boom, the available water in Gatún Lake is facing severe strain to meet drinking water requirements, Canal operations, and generating hydropower. The shortage is accentuated by leaky infrastructure that wastes close to 40-50% of municipal supply. Since the Canal is a critical maritime trade route, danger to its operations has a direct bearing on the world economy. Hence, it is important to look at the confluence of factors that have drastically reduced water availability, and which threaten both Canal operations and regional water security.

(a) Prolonged drought conditions. October 2023 was the driest month since the 1950s with recorded rainfall being 43% below the monthly average. The whole of 2023 was one of the two driest years in over seven decades and received an annual rainfall close to 20–30% below normal levels [17]. Although 2023 was one of the worst years, a cumulative water deficit severely impacted recovery of the main reservoirs. That points to a broader and worsening pattern of hydrological stress. Reduced precipitation in 2015-16, saw water level in Gatún Lake at 78.3 feet, nearly four feet below acceptable levels. In 2019, reduced water levels because of drought forced the Canal to impose shipping restrictions, reducing cargo loads, and increasing transit fees by up to 10% to manage congestion and costs. In 2023, even lower levels were recorded.

This reduced rainfall trend was compounded by rising regional temperature. Between 1980 and 2024, average temperatures in Panama have increased by approximately 1.1°C, accelerating rates of evaporation from reservoirs and reducing soil moisture retention [15]. A combination of insufficient rainfall and higher temperatures ensured that recovery of water bodies was slower even during the rainy season. These sustained drought conditions, coupled with watershed degradation caused by deforestation, significantly weakened the region's hydrological resilience.

(b) *El Niño and Climate Change Amplification.* One of the main causes of these critically low water levels was a significant deficit in rainfall during the 2023 rainy season (May–December), which sharply reduced the volume of water in Gatún Lake. Although water levels were above average in early 2023, a delayed rainy season with reduced precipitation caused by a shifting weather pattern resulted in the deficit. Such shifting patterns are strongly linked to the El Niño Southern Oscillation (ENSO) that typically brings drier conditions and shortened rainy seasons. Such a deficit was observed even in 2019. In 2023 it lasted longer till early 2024. Such climate drivers are slowly drying the Canal and making rainfall patterns in the region increasingly erratic. Climate models project that global warming is likely to make El Niño events more frequent and up to 15–20% stronger under high emission scenarios. This would eventually jeopardize the water stability and operational reliability of the Canal.

(c) *Deforestation in the Canal Watershed.* Widespread deforestation within the Panama Canal watershed has significantly contributed to declining water availability in the Canal's reservoirs. Forests in this region serve as natural water regulators by promoting infiltration, stabilizing soil, and maintaining the hydrological balance essential for feeding Lake Gatún and Lake Alajuela. This balance has been disrupted by decades of deforestation for agriculture, cattle grazing, urban expansion, and infrastructure projects. The Panama Canal watershed has lost nearly 20% of its forest cover between the 1970s and early 2000 [18]. This reduction has greatly diminished the landscape's capacity to retain and gradually release rainwater. In deforested areas, precipitation tends to run off rapidly rather than seeping into the ground, resulting in increased surface runoff, erosion, and evaporation [19] while increasing sedimentation in the Gatún and Alajuela Lakes, thereby reducing their effective storage volume and lifespan [20]. Thus, groundwater recharge and replenishment of critical freshwater reservoirs has reduced. Even during periods of normal or above-average rainfall, much of the water bypasses the Canal's storage system, reducing its capacity to sustain Lock operations [2].

Impact of a drying Panama Canal

A drying Panama Canal has profound implications at both the local and global level as it will disrupt maritime logistics and trade. A singular event of 2023 reduced daily vessel passages from 38 to 24 and resulted in delays, rerouting, and increased shipping costs. With climate change events more likely in a ‘high emission scenario’, the probability of the Canal drying increases multifold. Economists estimate that such disruptions could raise global shipping costs by up to 20% on certain routes and reduce Panama’s GDP by 0.1–0.3% through lost revenue and congestion surcharges [21]. Local communities depending on the Canal for employment and economic stability face uncertainty as operations become less reliable. Environmental concerns also arise, as dwindling freshwater resources and ecosystem imbalances threaten biodiversity in surrounding regions. Without effective intervention, the drying of the Canal would have significant consequences, ranging from economic losses to environmental degradation and social instability. Let us examine these impacts at the local and global level in greater detail.

Local Impact

Declining freshwater in the watershed of the Panama Canal severely affects local communities by disrupting agriculture, access to drinking water, and community stability. Over 2 million people (half of Panama's population) rely on Gatún Lake for drinking water. A shortage of drinking water heightens health risks and forces rationing of this precious commodity both in urban and rural areas. It would increase forced migration resulting in economic isolation and reduced economic gains from tourism tied to the Canal.

(a) Water Shortages for Local Communities. The Panama Canal requires approximately 200 million litres of freshwater for a single full ship transit. This requirement is met mainly by Gatún Lake and Alajuela Lake which together supply over 95% of the Canal’s operational water needs [22]. With an average of 35–40 transits per day over 7 billion litres of freshwater is used daily which is equivalent to the daily water consumption of the entire population of Panama. During drought years, this demand competes directly with local and agricultural water needs, creating an unsustainable pressure on limited reservoirs and exposing the Canal’s vulnerability to rainfall deficits [23]. When water levels drop, authorities prioritize the Canal’s functionality over local needs as the Canal generates over \$2.5–4 billion annually in tolls which is nearly 6–8% of their GDP. Low water levels in 2023–24 resulted in revenue losses of approximately \$600–800 million, dwarfing local agricultural impacts and prompting global transit cuts [24].

(b) *Economic Decline and Job Loss.* Panama's economy greatly depends on activities related to the Canal that include shipping, logistics and trade services. The revenue generated is through port operations, bunkering, transportation, toll revenues, and supporting jobs in sectors of piloting, port operations, ship maintenance, customs handling, freight forwarding, and warehousing. Such activities create opportunities for associated industries like banking, insurance, telecommunications, logistics providers, fuel suppliers, local shipping agents, tour operators, and catering services making the Canal the backbone of Panama's service-driven economy [25]. A reduction in daily vessel transit leads to decreased revenue and job losses in sectors tied to these maritime activities causing economic downturns in these sectors and those of port agency fees, eco-tours, and crew provisioning. Reduced water availability has resulted in fewer port calls, decreased cargo throughput, and less demand for ancillary services, particularly in key economic zones like Panama City and Colón. According to the Panama Chamber of Commerce, several small logistics firms reported a 15–25% drop in revenue during the second half of 2023, and local vendors and subcontractors near port areas experienced declining ship traffic and service demand [26]. This cascading effect shows that the Canal is both a maritime corridor and a lifeline for Panama's urban service economy.

(c) *Environmental Degradation.* The water crisis has significantly aggravated environmental degradation within the Panama Canal watershed, with clear and measurable consequences. Fluctuating water levels, caused by erratic rainfall, over-extraction, and soil's limited ability to retain water have disrupted the ecological rhythms of freshwater habitats. Fish spawning cycles, amphibian populations, and riparian vegetation are particularly vulnerable to sudden changes in water depth and flow velocity. Species such as *Atelopus zeteki* the Panamanian golden frog (actually a toad) and certain native fish like *Aequidens coeruleopunctatus* have seen marked habitat contraction [27]. The resulting biodiversity loss not only threatens ecosystem health but also weakens the watershed's natural resilience against further climatic extremes. These environmental stresses could undermine the Canal's long-term operability, as ecological degradation further limits water availability and storage [28].

(d) *Displacement of Local Communities.* To address freshwater shortage, the Panama Canal Authority (ACP) has proposed large-scale infrastructure projects, such as new reservoirs in Río Indio and Bayano basins to ensure operational stability for the Canal. These projects would have a high social cost as they would displace over 4,500 rural and indigenous residents including members of Ngäbe-Buglé and Emberá communities [29]. Since these communities depend on traditional farming, artisanal fishing, and culturally rooted land practices, relocating them is not just an economic disruption but a deep cultural loss. The proposed reservoirs would submerge

thousands of hectares of forest and farmland, threatening both biodiversity and livelihoods. Inadequate consultation and limited compensation have already provoked local resistance and protests. Displaced families often struggle to adapt to urban environments, leading to rising informal employment, housing insecurity, and social exclusion. This all deepens urban poverty and inequality, highlighting the complex trade-offs between environmental engineering and social justice in Panama's efforts to secure water for the Canal [30].

(e) Impact on Agriculture and Food Security. With freshwater resources being diverted to sustain Canal operations, agricultural productivity in the region has been severely affected. Many farmers depend on consistent water supply for irrigation but declining water availability has resulted in lower crop yields, increased food prices, and financial instability for local agricultural workers [16]. The reduced harvests not only impact local food security but also affect Panama's ability to meet domestic demand and increasing dependence on imported goods. This shift places additional economic strain on lower income populations who already struggle with rising costs of essential goods.

Global Impacts

Disruption in movement across the Panama Canal caused by droughts has reshaped global supply chains by spiking shipping costs by 15-40% on key Asia-US routes and delaying commodities like LNG, grains, and containers [31]. Such changes have forced nearly 40% of the transits to be rerouted around South America. As a result, supply chains have been disturbed and have created inventory shortages in retail and automotive sectors. The drought of 2023 alone impacted approximately 5-7% of world trade connecting 170 countries [32].

(a) Disruptions to Global Trade and Supply chains. The Panama Canal facilitates nearly 6% of global maritime trade between the Atlantic and the Pacific Ocean [32]. The operational capacity of the Canal will be significantly reduced if current weather trends continue. Since modern vessels are larger, they require more freshwater for each crossing. Because of reduced water levels during droughts in 2023 the Panama Canal Authority was forced to reduce daily ship transits to 24 from the earlier 36-38 forcing many shipping companies to seek alternative mechanisms such as impose cargo limits, reroute vessels, or increase freight charges. This resulted in widespread disruptions to global supply chains and brought about price volatility, delivery delays, and logistical bottlenecks across major markets [33].

(b) Rising shipping cost and Alternative routes. Consequent to drought in 2023, the Panama Canal forced transit caps. Although it was anticipated that transits would return to full capacity by September 2025, recovery has been uncertain. In the first quarter of 2025, only 13 LNG (liquefied natural gas) vessels used the Canal and transits of dry bulk carriers dropped by 35% to 129. By the end of 2025, although the number of transits exceeded those in the previous drought period, driven by risks in using the Suez Canal, they were still lower than their averages [34]. So far, container vessels have maintained normal usage levels; but the decline in energy and bulk commodity transport shows the growing fragility of global supply chains amid climate driven disruptions. With transit restrictions in place, shipping companies are forced to use alternative routes such as the Suez Canal or around the southern tip of South America (Cape Horn). These options increase cost towards fuel and crew, transit times, and carbon emissions as was seen even for ships moving from Europe to Asia after the Houthi attacks in the Red Sea [35]. The rise in operational expenses is subsequently passed on to the consumers resulting in global inflationary pressures. This will be seen clearly when the March 2026 Israel and U.S. attacks on Iran are fully assessed in economic terms. Sectors that rely on just-in-time logistics, such as electronics and automotive industries, are especially vulnerable, and smaller businesses face mounting challenges in inventory management and meeting customer demand. Such shipping uncertainties have led to stockpiling, straining warehousing and logistics capacity, and increasing insurance premiums for extended voyages. Without a sustainable and climate resilient water management strategy, continued restrictions on Panama Canal transits may permanently alter trade routes and accelerate a shift toward more regionalized supply chains.

(c) Geopolitical and Economic Consequences. Drying of the Panama Canal has broader geopolitical implications, as nations dependent on this trade route seek alternative solutions. Countries with major exports moving through the Canal, such as China and the US may shift trade agreements, invest in infrastructure projects, or explore new logistical hubs. Additionally, competition among global trade routes, such as the Arctic shipping lanes, which are becoming more navigable through increased global heating, could reshape international shipping patterns in the coming decades [36]. The disruption also strengthens the strategic importance of other major canals and ports, such as the Suez Canal and encourages developing rail networks connecting Asia and Europe such as the Belt and Road Initiative (BRI), India Middle-East Europe Corridor (IMEC), Asia-Africa Growth Corridor (AAGC), and the International North-South Transport Corridor (INSTC). Industries reliant on just-in-time shipping may accelerate investments in regional manufacturing and supply chain diversification to mitigate future risks. Furthermore, alliances and trade partnerships may shift as countries seek reliable and cost effective alternatives for transporting goods. The economic strain on Panama itself could prompt increased foreign

investments or influence from major global powers with an aim of greater control over critical trade infrastructure.

(d) Strain on International Energy Markets. The disruption of Panama Canal transit has significantly impacted global energy supply chains, particularly for LNG and petroleum exports. Many energy shipments rely on the Canal for efficient transportation between major producers and consumers, including the US, Asia, and Europe. As delays force tankers to take longer routes, fuel supply uncertainties contribute to increased volatility in global energy prices. Rising transportation costs are passed on to consumers and industries, leading to higher electricity and fuel prices worldwide. Additionally, energy dependent economies face heightened risks of supply shortages, prompting governments to reconsider energy security strategies and diversify sourcing options. Some countries have begun stockpiling fuel reserves to cushion against potential disruptions, further driving up demand and market speculation. In the long term, sustained challenges in future Canal transit could accelerate investments in alternative energy sources and infrastructure to reduce reliance on fossil fuel imports. The rising cost and use of alternative routes as discussed earlier will add further to the existing strain on international energy markets.

Discussions and Way Ahead

The Panama Canal was built in 1904 as a means to move the US naval fleet between the Pacific and the Atlantic Oceans in quick time. After the Second World War, while the US continued to control the Canal it was opened to commercial ships, mainly the US ships, as a shorter trade route to Asia. It was only in 1999 that the Canal came under the full administrative control of Panama. Since then, the 82 km Panama Canal has been instrumental in transporting 5-6% of global maritime commerce annually in a cost effective and time saving manner. The operation of the Canal requires approximately 200 million litres of freshwater for the transit of a single ship which is supplied mainly by Gatún Lake and Alajuela Lake. Along with this requirement, these two lakes supply fresh water to the population of Panama while being dependent on rains for fresh water replenishment. In the event of a drought, as experienced in 2023, the lakes will show a major fall in available water levels. As in 2023, this event will, again, force authorities to reduce the number of transits.

This water shortage has had an impact both at the local and the global level. For the local communities, drinking water shortage became a major issue as Canal operations were given priority over supply of water to the residents. With the Canal supporting nearly 6-8% of their GDP, reduced transits impacted the GDP of Panama and the spin-off jobs associated with these transits. Over the

years, environmental degradation caused by deforestation and climate change events have reduced precipitation levels in Panama resulting in drying up of the lakes that support the Canal's operation. This will compound the problem. Agriculture will be affected by the reduced capacity to retain and gradually release rainwater. Again, there will be rapid runoff, erosion and evaporation of rain water rather than seeping into the ground.

Although deforestation has continued since the 1950s, the impact was realized only in 2023 when a severe drought reduced the water level of the lakes to minimum permissible levels. Since such events are an alarm, it is important that Panama makes efforts to conserve and protect their natural environment to minimize any effects of a future drought. Studies indicate that climate change events are likely to increase under high emission scenarios or the business-as-usual scenario. The Panama Canal thus requires a combination of environmental restoration, technological innovation, and policy reforms. This requires immediate and long-term measures to ensure sustainability of the Canal and its surrounding ecosystems while maintaining the economic stability of the country. Without proactive intervention, the water crisis in Panama will continue and threaten local livelihood, regional economies, and global trade. An integration of scientific research, stakeholder collaboration, and adaptive management strategies is thus essential for developing effective solutions. Securing adequate funding for conservation projects and infrastructure improvements is essential to implement these measures successfully. Some possible mechanisms are recommended for consideration.

(a) Large Scale Reforestation and Watershed Protection. Reforestation in the Panama Canal watershed is considered the start point to restore the region's natural ability to retain water. Expanding protected forest areas, enforcing stricter deforestation laws and giving landowners incentives for sustainable land use practices can significantly improve water retention and reduce soil erosion [37]. For example, the tourism industry in Panama should be encouraged to focus on these realities. Afforestation programs need to plant native tree species to enhance biodiversity and stabilize the ecosystem. Additionally, educating local communities about sustainable agricultural and forestry practices can prevent further land degradation. Local communities who adopt environment-friendly conservation practices need to be provided with financial incentives. Research institutions and environmental organizations need to be encouraged to assist in developing reforestation models tailored to Panama's unique climate conditions. By restoring forests and preventing further deforestation, Panama can create a more resilient watershed that supports both the Canal and local communities.

(b) Improved Water Conservation and Management. Adoption of advanced water saving technologies can optimize freshwater used for operations of the Canal. Renewing infrastructure to

minimize water loss through leakage and evaporation is crucial. Smart monitoring systems can be introduced to track water levels and predict shortages, allowing authorities to implement proactive measures [38]. Using digital twin technology, virtual simulations of real world infrastructure, can help optimize water management strategies [39]. Such efforts can be accelerated with the participation of private players either in a public-private partnership model or through full ownership by the private players.

(c) Development of Alternative Water Sources. Investing in rainwater harvesting systems can supplement freshwater availability for the region. Additionally, innovative water extraction methods, such as atmospheric water generators, could provide sustainable long term solutions. This needs to be adequately supported by community based water conservation programs to help distribute resources more efficiently, so that there may be equitable access to all. There needs to be also the use of enhanced groundwater recharge techniques to undo the diminished capacity of the soil to retain and release water. The integration of multiple water sources will create a more sustainable and adaptive system for managing water availability in the region. Although some may even recommend desalination plants to meet the water requirement of the country, they are not recommended as desalination plants have their own ecological concerns [40].

(d) Increase holding basins for recycling water from Locks. To reduce fresh water consumption from the lakes requires a comprehensive design that would need building new basins and integrating existing gravity-fed basins with active pumping. For the Neo-Panamax Locks, nearly 60% of water is recycled by using three basins created adjacent to the Lock (see Fig. 2). To ensure more water can be recycled, the existing basin system will need to be modernized to five in place of the existing three. For the older two-lane Locks, although nearly 50% of the water is recycled between parallel lanes (see Fig. 2), to save the remaining 50% water which is discharged into the ocean, new basins will be required wherein the water can be pumped into and from the Locks to minimize the demand of freshwater from lakes. This development must be preceded by a feasibility study. The existing water transfers are done using gravity without using power, but these proposed basin transfers will require mechanical pumping systems. Although these electrical pumps will be energy-intensive, the revenue from a transit greatly exceeds the cost of operations. The operations of these pumps can be made sustainable if operated with electricity produced from renewable sources. Although the entire transit per ship may increase, this sustainable development will ensure that the Canal's operations will continue even if droughts are experienced by the region. Higher availability of fresh water in the lakes will ensure the well-being of Panama's population and reduce migration, providing more economic activities in Panama and hence greater economic prosperity for the country. With 100% recycling, implementing reverse osmosis or multi stage flash

desalination plants at the lock interface will need to be established to avoid salt water intrusion into the freshwater ecosystem of Gatún Lake.

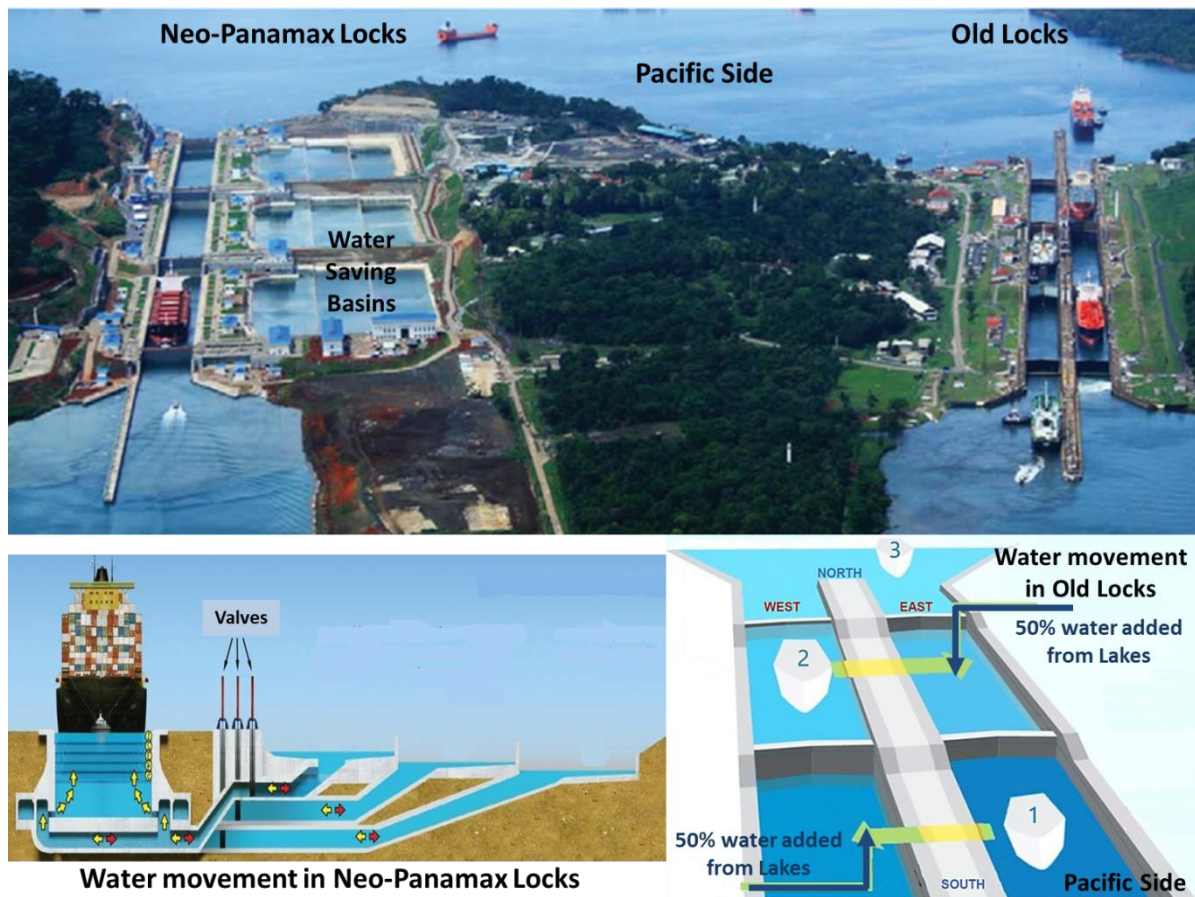


Figure 2. Water movement in Old and New Locks (Source: Authors, from various resources)

(e) Recycling Grey Water for reduced dependence on fresh water. Panama City lacks a centralized municipal grey water recycling programme for individual homes and hence implementing an onsite solution to address this issue will reduce the annual water consumption of the city. Mechanisms such as nature based solutions (NBS), mechanical filtration, biological treatment, and direct diversion are some common recycling methods that can be explored. Such treatment systems can be implemented by means of local service providers.

(f) Policy Reforms. Government policies are essential to prioritize sustainable water management through stricter environmental regulations and better resource allocation. This needs to be adequately supported with transparency in water governance, including public access to water usage data and decision making processes for greater accountability. Businesses could be persuaded to adopt water efficient practices through the incentives of tax benefits or subsidies. Participation in conservation efforts needs to be nation-wide. Strengthening the legal framework to end deforestation and illegal land exploitation is essential to avoid further degradation of the forest

cover. To adopt and introduce advanced technologies for conservation, international collaboration with trade partners and environmental organizations may be encouraged.

(g) *Creating climate change resilience.* It is considered essential to include in Panama's national policies the components of an integrated plan for climate change resilience through national adaptation, improvements in infrastructure, the use of nature based solutions, and an openness to data as they emerge, to maintain flexibility in policy for the sake of long term stability.

Conclusion

Drying of the Panama Canal is a multifaceted crisis driven by climate change, deforestation, and poor water management. The consequences extend beyond Panama, affecting global trade and regional livelihoods. Immediate and long term strategies, including reforestation, improved governance and sustainable infrastructure, are considered imperative. Strengthening international cooperation and fostering innovation in water conservation technologies will be a key to mitigate the existing crisis. Additionally, proactive policy reforms and economic diversification will help reduce Panama's vulnerability to future water shortages. These challenges require a holistic response that integrates environmental, economic, and social considerations. Without decisive action, the Canal's long term viability and its role in global trade will remain at risk. The present research has endeavoured to discuss the causes, impacts, and mitigation measures. The recommendations offered here integrate solutions that balance environmental conservation with economic sustainability. It is essential to include government agencies, private enterprises and local communities in the attempt to maintain the Panama Canal in a functioning state.

Although the construction of the Rio-Indio reservoir of the Panama Canal Authority approved in February 2025 is a long-term water security strategy which will ensure a minimum of 36 daily transits even during severe dry seasons and could potentially add up to 15 additional ship transits per day once fully operational, it faces serious property appraisals and community resettlement plans [41]. Nevertheless, ensuring resilience depends on stopping deforestation and coping with climate change. By integrating measures discussed in the paper, Panama can mitigate the Canal's water crisis, ensuring long term economic stability and environmental sustainability. Otherwise, the economic and geopolitical significance of the Panama Canal may decline, leading to long term global trade disruptions. Strengthening Panama's resilience against climate change will serve as a model for other water stressed regions worldwide. Investing in long term sustainability will not only benefit Panama but also secure stable maritime trade for generations to come and hence must be undertaken on a near war-footing.

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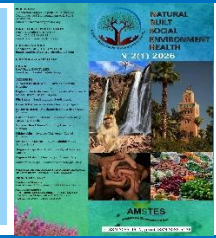
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Rare Events: Are Outliers Negligible?

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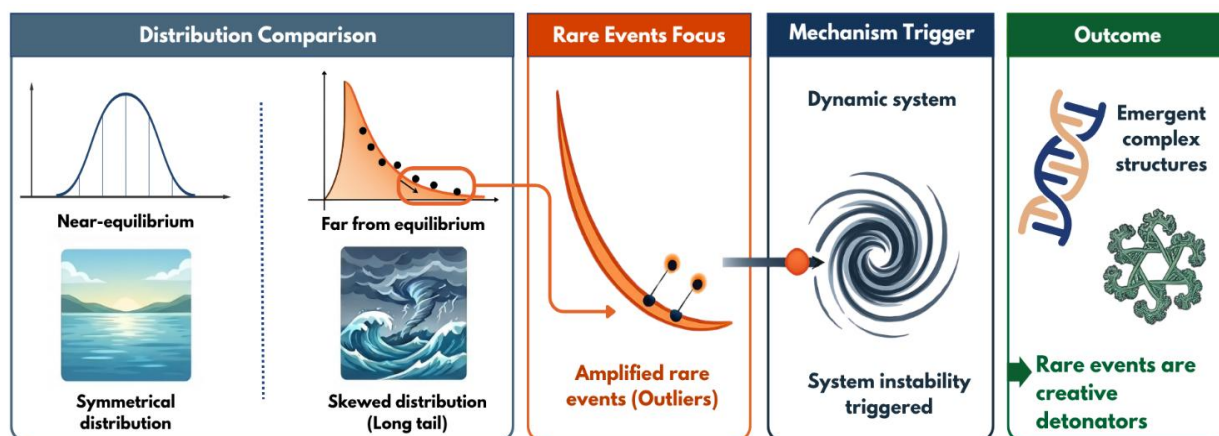
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Graphical Abstract

Do rare events sit at the margins, or do they shape the course of complex systems? As density falls and symmetry breaks, these overlooked “outsiders” emerge, revealing how fluctuations and asymmetry reorganize behaviour beyond the average. Far from equilibrium, such rare trajectories are amplified, triggering instabilities that quietly steer systems toward new, often unpredictable paths.



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Abstract: Do rare events merely sit at the margins of statistical distributions, or do they shape the evolution of complex systems? This work explores how asymmetry and fluctuations, especially in non-equilibrium conditions, redefine system behaviour. Numerical simulations show that as particle density decreases, distributions become increasingly skewed, revealing the growing role of extreme, low-probability configurations. These “outliers”, often overlooked in favour of mean behaviour, emerge as dynamically distinct components. Through entropy analysis and simple interaction models, the study demonstrates how local fluctuations and initial heterogeneities can lead to structured, asymmetric organizations. These effects extend beyond physics, offering insight into biological and systemic transformations driven by rare configurations. A simplified electro-driven molecular model further illustrates that rare trajectories can trigger nonlinear instabilities, leading to trapping, convection, and enhanced transport. In such regimes, fluctuations are amplified rather than damped, reshaping global dynamics. Overall, rare events are not negligible anomalies but active drivers of system evolution, steering complex systems beyond predictable averages.

Keywords: *Rare events, Asymmetry, Dynamics of out-of-equilibrium systems.*

Time's Arrow and Thermodynamics: A Taboo?

The question of time's direction has sparked endless debates and will likely continue to do so. More precisely, its link to thermodynamic irreversibility deserves close scrutiny. Although time is not explicitly part of thermodynamics' laws, the second law states that every process must increase global entropy. Jill North [1] explains that in a system like a gas of randomly distributed particles—macroscopically described via Boltzmann's statistical framework—extreme fluctuation domains can show localized entropy decreases, even as the system's overall entropy rises. Thus, time's evolution towards higher entropy does not rule out regions of major deviations¹ where disorder (entropy) is greatly reduced. According to Harold Blum in *Time's Arrow and Evolution* [2], the inexorable thrust of time—embodied in the second law of thermodynamics and its decree of rising entropy—serves as the cosmic compass guiding life's unfolding drama. From the universe's primal forge, physical and chemical edicts have carved narrow channels for evolution, curtailing possibilities long before life's tentative spark ignited.

This shifts us from time's arrow to what rarity, grasped in time, truly adds—or subtracts—from the extraordinary. First, what do we call a “rare event”? Common sense says it is any event with a near-zero probability. Yes—but who is asking?

Imagine a fund manager handling Euromillions: twice weekly, 104 times a year, he transfers 20–200 million euros (or the equivalent) to some winner. This has been routine since February 2004, when the Lotto game was launched.

Now picture a retired winemaker from Lézignan-Corbières winning 70 million. Life and family are upended—how could they foresee being the first winner in their department after 22 years? Odds: 1 in 113,983,816!

Does this not prompt reflection on what one sees as rare or improbable? Perspective matters.

In the Big Bang model, as the early universe evolved, tiny initial variations in particle densities were amplified by gravitational collapse—a self-reinforcing process that pulls in more and more particles, forming increasingly dense clumps. Similarly, the Janus model [3] suggests that “negative” and “positive” particles attract those of the same type and repel opposites, sparking the creation of both densely packed regions and vast empty voids (such as the Great Repeller). These mechanisms show how systems can develop areas with extreme deviations, whose traits starkly

¹ supporting therefore very unlikely trajectories.

contrast with the overall probabilistic system, where fluctuations remain small and near the average. Some even propose that Earth—or any local structure, like a tree leaf or a human body—could be such a region, evolving towards lower entropy relative to the whole universe. This essay explores to what extent systems, like cooling gases, can produce these domains, and whether such rare small-scale setups might give rise to highly improbable complex structures. Anisotropy, fluctuations, and emergence arise together as seeds of the dynamics we observe in the universe. Physics teaches us that near a critical point—like the phase transition from liquid to gas—fluctuations become macroscopic and pivotal: a small one can take over the entire system, reshaping its global properties. It often seems the same holds true (at times? often?) for matter and human affairs

Tails of Fate: How Pareto Forges Asymmetry

To simulate a cooling gas, we use a simplified numerical model. This model features an under-sampled universe of constant volume (represented as a 2D surface) where particle density decreases as the universe expands and cools. The simulation has two parts:

- Non-interacting particles: no interactions (e.g., gravitational forces) are considered.
- Mass–mass interactions: a basic approximation of particle–particle attraction is introduced.

In the first part, the simulation iteratively generates random particle positions using a pseudo-random number generator. The number of particles N varies logarithmically from 30 to 10,000 (10^4), with 100 discrete steps. For each N -particle setup, 30 random iterations calculate nearest-neighbour distances, with the random seed changed each time. The skewness² of the resulting spatial distribution is averaged over iterations, and the standard deviation is computed. We then analyse the evolution of skewness versus particle density³.

In the second part (see Appendix), the system is modelled on a 32×32 grid. Temporal entropy evolution is quantified using a simplified mass–mass attraction scheme (details below).

The first simulation's result appears in Figure 1, showing skewness evolution as particle density drops—as in universe expansion and cooling. The arrow of time points rightwards on the graph. Red dots show averages ($n = 30$) $\pm$ standard deviation in blue. We first note that average skewness

² *Skewness* indicates whether the data are spread out more on the left or on the right side of the average, instead of being evenly distributed.

³ The simulations are run in the R statistical environment.

stays above 0.5 in all cases, even though a large population ($N \gg 0$) should trend towards a normal, purely Gaussian distribution (the perfectly symmetric bell curve centred on its mean), with zero skewness. This stems from the lower bound constraint on particle encounters: distance can be zero but not negative.

As particle density falls from 10^4 to 10^3 , skewness rises, along with the spread of values, as shown by the growing standard deviation (blue bars in Fig. 1). Overall, this means that “outliers”—particles in sparse space—become proportionally more common relative to the total number of particles at low density, with skewness rising exponentially at the lowest densities, especially for $N = 30$ in this case: at very low density, skewness exceeds 1, a value typically signalling a highly skewed distribution

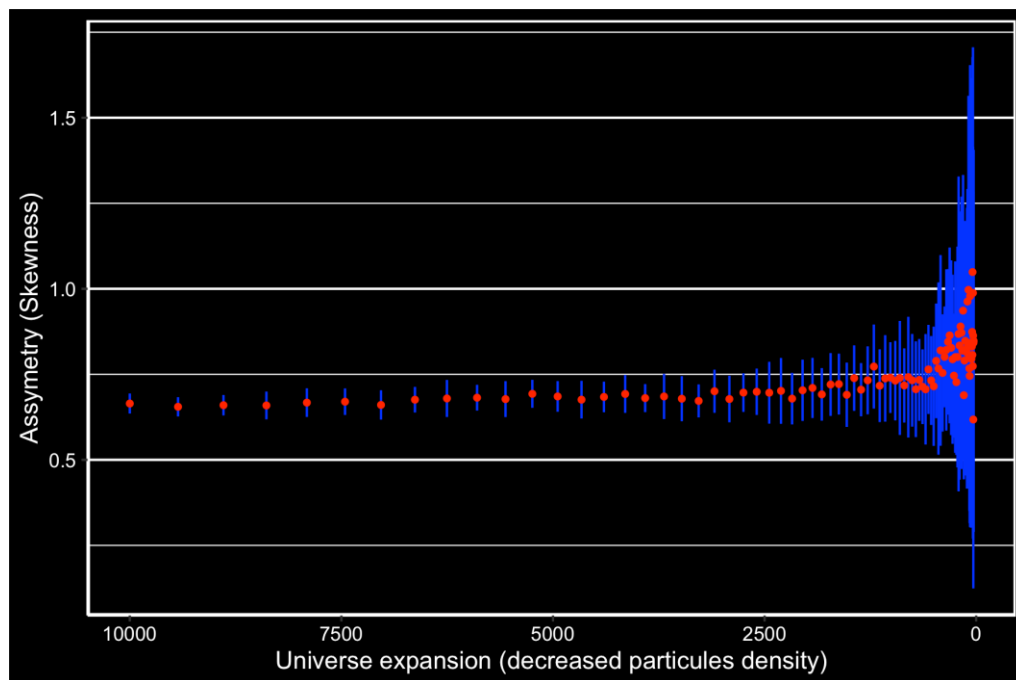
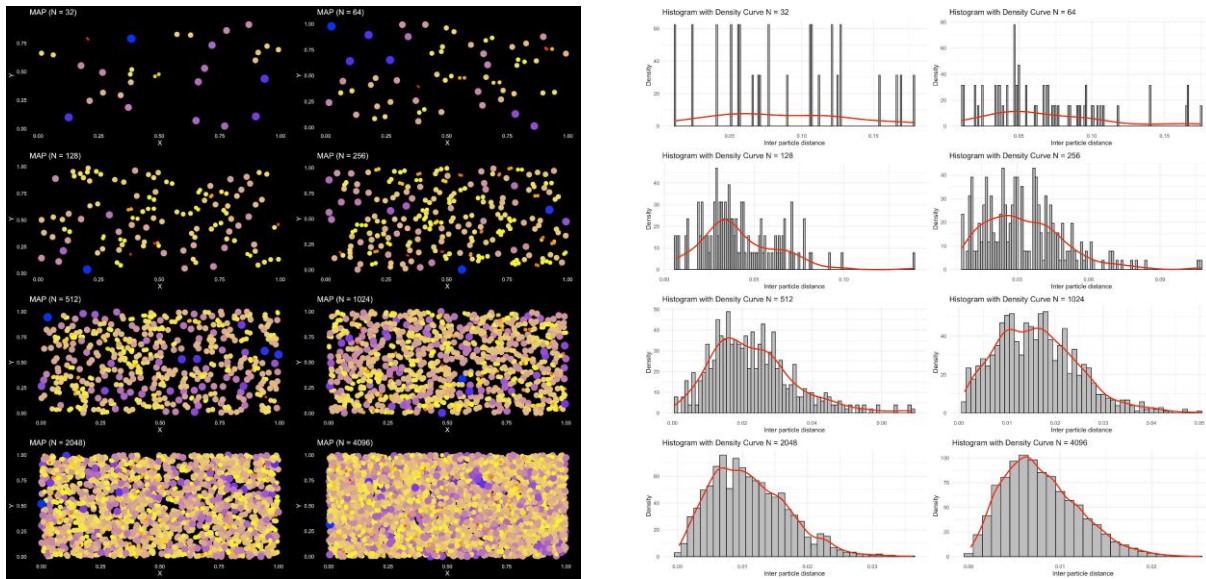


Figure 1. Evolution of skewness as particle density decreases

To illustrate better skewness evolution as particle density changes, Figure 2 shows spatial distributions and histograms of nearest-neighbour distances for random simulations with $32 \leq N \leq 4096$. Density curves (in red in Fig. 2b) help to visualize the asymmetry of the corresponding distributions. For example, skewness rises from 0.67 to 1.11 as the number of particles N drops from 1,000 to 100. Note that the parameter here is the minimum distance between two particles. In Figure 2a, particle colour and size encode their nearest-neighbour

distance: smaller size and redder colour mean that particles are closer to another particle; larger size and bluer colour mean that particles are farther from all others.



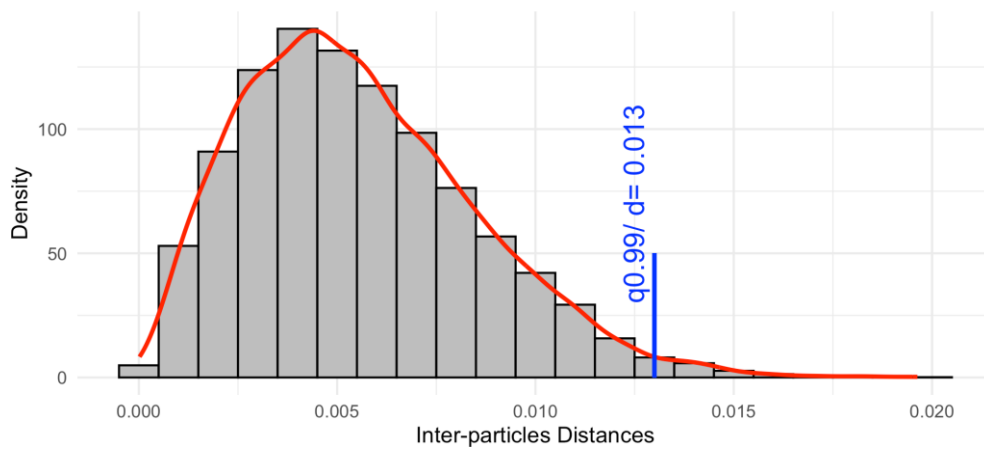
(a) Random spatial distributions

(b) Nearest-neighbor distributions

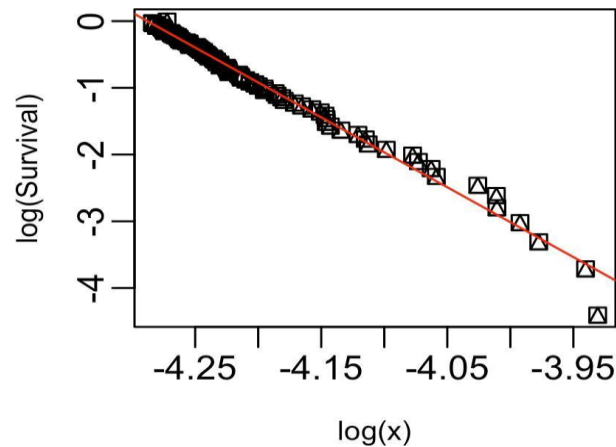
Figure 2. Initial distributions ($32 \leq N \leq 4096$)

To characterize better the long tail of the nearest-neighbour distance distribution, we analysed the case $N = 4096$, $n = 8192$, which yielded a skewness of 0.686. We tested whether this distribution exhibits a heavy tail that can be fitted by a Pareto law, which would more precisely describe its asymmetry. The data were analysed using the R package ExtremeFit [5]. This package first identifies an adaptive threshold τ beyond which the data appear to follow a Pareto distribution, then tests the goodness of fit for the data points beyond this extreme quantile ⁴.

⁴ The hill.adapt function returns $\tau = 0.0139$ (HASXadapt), the literature's adaptive threshold from which the tail can reasonably be modeled as Pareto-distributed



(a) Minimal distances distribution



(b) log–log plot for the 0.99 extreme quantile

Figure 3. Pareto evaluation for $N = 4096$ and $n = 8192$

Thus, Figure 3a shows the 0.99 quantile of this distribution, indicating that a second approach was developed to quantify entropy variations over time (Appendix A).

What Have We Learned So Far?

Do outliers matter? That is the question this essay aims to answer, with some thoughts on their possible consequences. In science, what catches the eye in an observed distribution is usually its first two moments: the mean and standard deviation (their position and uniformity). Tails—left or right—are often overlooked and attributed either to measurement errors or to individuals not worth a second glance. One exception: security services, which actively zoom in on rare, unexpected human behaviours spotted by surveillance cameras— “the exception” becomes the algorithm’s

target! The perspective shifts when we ask how these outliers evolve over time compared to those near the mean, for example within two standard deviations and thus representing 95% of the population ($\bar{x} \pm 2\sigma$). Do the “mainstream” truly rule the world? Should we pay attention to rare events?

In this simulation, a particle may represent a simple gas molecule or a small world, small compared to the size of the system, which is vastly larger than that of the “particle” itself, whatever its representation or kind⁵... At all time scales, gravitational forces or any other type of interaction are responsible for the spatial fluctuations of the particles, whether or not they collide. As mentioned above, any distribution observed at a given instant t serves as the starting point for the subsequent dynamics. The origin of the time arrow is itself dynamical! It is well known that very similar initial conditions can later give rise to drastic divergences, characterized by the Lyapunov exponent⁶. This is because the probability of a particular event, for example rolling a six on a die, does not depend on the previous roll⁷.

Since this approach stems from asymmetric distributions—even when $N \gg 0$, which is a priori unexpected—we must consider both the “left” and “right” tails of the density curves. Here, skewness is always positive and above 0.5; asymmetry is thus more pronounced on the “right” side than on the “left” side of the bell curve. This means that particles with large distances to their nearest neighbours outnumber those forming clumps. We shall call these the “right” and “left” cases. The “right” case—the famous heavy tail—groups particles (or tiny solitary worlds) that interact weakly with the system, since interactions between two entities classically decay as the inverse square of their separation distance. This means that they are barely exposed to external forces, contributions, or noise, evolving quasi-autonomously—based mainly on their internal forces⁸.

At the opposite end, the “left” case—less populated but real (see Fig. 2a), especially in a hot universe—exists. Unlike the previous case, these particles or small worlds face external forces from nearby neighbours: they act on their closest neighbours and at the same time are exposed to their

⁵ Here a “particle” is understood as an object without any particular spatial extent.

⁶ A Lyapunov exponent measures how quickly two trajectories of the same system, starting from nearly identical initial conditions, separate from (or approach) each other over time.

⁷ In mathematics this is the opposite of Markov chains observed in many different contexts ; one then speaks of independent events.

⁸ We consider here that a particle can represent a complete little world of its own, subject to its own laws regardless of its nature, a sort of scale-free entity.

effects. This status is the opposite of that of distant particles or worlds. Picture the galactic centre, where stellar density is huge: Proxima Centauri, the Sun's nearest star, lies 4 light-years away, whereas at M32's core—the “compact dwarf galaxy”—stars are just 3 light-days apart! We must admit that these two types of subsystem show very different dynamics

These distinct dynamics for both statuses can be illustrated, in my view, by what's called conformational drift in recombinant proteins [6]. These proteins are “artificially” synthesised by bacteria or yeast after targeted genome modification (GMOs ⁹). Specifically, these are dimeric proteins—made of two distinct polymeric chains that assemble post-synthesis to become biologically functional. If recombinant chains are synchronously synthesized, they assemble correctly. Conversely, if a time lag exists between the two chains' synthesis, the first begins relaxing: its conformation evolves through interactions with solvent molecules, its nearest neighbours. As a result, the second chain—synthesized later—no longer recognizes its partner, and the functional dimer never forms. This is called conformational drift. Such protein misfolding causes many genetic diseases, including cystic fibrosis.

Let us return to high-deviation regions. It is very likely that they exist and evolve differently depending on whether they form a sparse environment (“right” case) or a highly dense one (“left” case). One may wonder whether such distinct states have been observed in the universe. In fact, in the core of certain galaxies, the matter density is about $3 \times 10^{-14} \text{ kg/m}^3$, whereas in cosmic voids it drops to approximately 10^{-22} kg/m^3 . This eight-order-of-magnitude difference reflects, as mentioned above, the evolution of density fluctuations over time as the universe expands.

At this stage, one must admit that skewness arises spontaneously, and that large deviations can exist and evolve within the global system, without violating the probabilistic interpretation of the second law of thermodynamics¹⁰. Although they evolve towards opposite entropy values, their contributions to the global macrosystem remain, apparently, negligible given their rarity and their tiny spatial scale. We shall see that the time evolution of any system is governed by its network of interactions: even an infinitesimal initial difference can lead over time to systemic divergences that appear alien with respect to the dominant behaviours. Yet, these observed divergences ultimately stem from the intrinsic dynamics of out-of-equilibrium systems. If such microscopic worlds wished

⁹ Genetically Modified Organisms.

¹⁰ In fact, the second law of thermodynamics is not violated, since it applies only to macroscopic systems and not to micro sub-systems.

to communicate with similar entities, they would require infinite patience and the development of extraordinarily powerful, extremely long-range radio telescopes.

The Paradox of Infinite Improbability

Before discussing the role of these high-deviation regions in system and subsystem dynamics, let us quickly survey rare events that occur despite seeming nearly impossible. The “historical” field is full of such cases. According to Hannah Arendt [7], every human action appears as a miracle—that is to say, an infinite improbability that repeats. Beyond its own infinite improbability, the power of even the smallest event lies in its ability—even amid the most constrained circumstances—to change all combinations of circumstances. In modern history, or “modern science”, events arise unexpectedly, without roots in hidden underground currents. Historians strive to weave a “logical narrative” to explain them, yet no causal link fully accounts for their occurrence.

The most striking example is the origin of life. Hannah Arendt gets straight to the point: it is the infinite improbability of the event that breathes life into what we call the real. Following Arendt [7], the origin of life in matter may be understood as an expression of infinite improbability arising from inorganic processes, just like the origin of the Earth from the viewpoint of universal processes, or human evolution from animal life.

The new always clashes with the formidable probabilities of statistical laws which, under ordinary circumstances, amount practically to a certainty. This fundamental paradox—events arise without apparent cause—has major consequences for what we conceive as real, because our real is rooted in what happens, and what happens defies traceable origins despite its sudden emergence. That is where our “lifeline” of rationalism breaks: modern reason has become shipwrecked on the reef of the real [7].

Asymmetry and Chaos

Are we therefore led to a dead end? Certainly not if we now turn to the dynamics of non-equilibrium systems! The most fascinating system of this type is undoubtedly life. The dissipative structures highlighted by Ilya Prigogine [8] have definitively introduced bifurcation into our way of thinking about the future: a sudden, unexpected event that radically alters the space-time trajectory of any (sub)system. For Giuseppe Longo [9], historicity must be understood in terms of changes to the space of possibilities (phase space) as well as the role played by diversity in the structural stability of living systems and the role of rare events in

shaping history. On the stage of life, this places the “unpredictable” at the centre. Several studies have linked the thermodynamics of non-equilibrium systems (dissipative structures, chaotic systems) to asymmetric distributions. Overall, complexity studies rest on the role of such distributions in non-equilibrium steady states, entropy production, and symmetry breaking¹¹.

But we must be precise about what we are discussing. First, these systems—far from thermodynamic equilibrium—show particular behaviour. Their structure directly results from energy flowing through them. If the energy flux dries up, the structure disappears. In Northern Europe, we enjoy a temperate climate compared to North America at the same latitudes, thanks to the Gulf Stream—which climatologists now fear may end. Indeed, if this ocean circulation stopped or reversed, we would face the same harsh winters as New York.

Another trait is that these systems are extremely sensitive to even weak fluctuations. Small variations can suddenly spread through the entire system, steering it in a totally unexpected direction. This is the opposite of equilibrium systems, where a small environmental change is damped by the system’s components. A well-known effect in innovation networks concerns their resilience: a well-established network with many partners is less resilient than a “fragile” (but more agile) one, as it instinctively rejects the introduction of a new partner (here playing the role of perturbation or fluctuation) whose ideas and innovation seem out of frame or irrelevant.

Thus, near equilibrium, rare fluctuations cancel out statistically, and the system stays predictable around its mean. Far from equilibrium, continuously injected energy (e.g., wind for turbulence, food for life, innovation for networks...) amplifies these rare deviations, making them decisive for system evolution. Imagine a boat: in calm (equilibrium), rare waves do not matter; in a storm (non-equilibrium), an exceptional wave—a rogue wave—can change everything.

In living systems (non-equilibrium maintained by metabolism), rare genetic mutations or mass extinctions drive evolution by altering natural selection. They enable major adaptive leaps, such as new species emerging, because the system is far from equilibrium and sensitive to extreme deviations. Thus, life’s history often depends on these amplified “accidents” rather than gradual changes.

Take cystic fibrosis: this genetic disease stems from the deletion of three base pairs in the gene coding for the protein that transports chloride ions across cell membranes. Human DNA has about

¹¹ Place a perfectly sharpened pencil flat on a smooth round table. Theoretically symmetric, it can roll indifferently left or right. In practice, a tiny imperfection makes it tip to one specific side—the rotational symmetry breaks spontaneously, creating a privileged direction without apparent external cause.

3 billion nucleotide base pairs. Statistically, what weight should be given to a “mistake” of $1/1,000,000,000$? If we now consider the living conditions of affected patients, our viewpoint will certainly be entirely different.

In conclusion, non-equilibrium systems like life or turbulence do not trend towards a stable average state but constantly evolve through energy or matter exchanges with their environment. In these systems, rare events—low-probability but high-impact—often dominate overall behaviour, unlike equilibrium systems where the average suffices.

Prigogine’s foundational works—from his 1955 statistical mechanics of irreversible processes to his 1977 synthesis with Nicolis—show how dissipative structures emerge in systems far from equilibrium—like living cells or oscillating chemical reactions—by channelling energy fluxes towards dynamic order, well beyond static equilibrium [10-12]. Asymmetry plays a central role there: Lefever and Prigogine’s 1968 paper highlights instabilities that break symmetry, amplifying tiny biases into non-Gaussian asymmetric distributions that defy “bell curve” predictability and spawn unexpected temporal rhythms and spatial patterns [11].

Blum [2] wields “time’s arrow” not as evolution’s architect, but as its stern sentinel: entropy swells universally, yet local eddies of order—negentropy—arise through energy fluxes, yielding complexity without defying the grand dissipation. Blum describes life as a dynamic steady state that is not thermodynamic equilibrium (where fluxes vanish) but a stable non-equilibrium regime with continuous dissipation. So, Blum and Prigogine converge on self-organization via irreversible fluxes.

Thus, chaos enters through fluctuating intermittency, where Glansdorff and Prigogine (1971) stress stability within flux, and rare events—sudden bifurcations—redirect trajectories unpredictably, echoing the improbable leaps of life highlighted in Prigogine’s 1967 thermodynamic essay on biology [13,14]. Together, asymmetry, chaos and rare, unpredictable fluctuations divert entropy’s course towards creative evolution.

Cross-Field Perspectives

We shall now try to understand how a system shifts from near-equilibrium to far-from-equilibrium states, and what happens during this transition. For this, we shall consider a very simple system: a protein molecule in solution subjected to an electric field between two electrodes—the anode (positive pole) and the cathode (negative pole). Most proteins carry a negative charge at neutral

pH. This simulation is adapted from Pérez-Cárdenas [15]. These more or less charged molecules will thus tend to migrate towards the anode through opposite-charge attraction ¹² (Fig. 4).



Figure 4. Migration of a charged particle in an electric field

In this system, two distinct forces act on the molecule. First, it is subject to Brownian motion: depending on temperature, water molecules jostle it in a disorderly manner, with speed and kinetic energy¹³ that increase with temperature. Think of bumper cars: no preferred direction exists, and molecules move randomly in solution as a result of these constant collisions.

The second force comes from the electric field the protein experiences because it carries an electric charge (negative in our example). This one has a defined direction: the protein migrates towards the opposite pole, and its strength ($F = qE$) depends directly on field intensity and the charge q carried by the protein.

For our demonstration, we shall consider motion along just one dimension (here the horizontal x -axis), although thermal agitation affects all three spatial dimensions, of course. The simulation experiment starts at $t = 0$ and runs for 10 time units without specifying whether these correspond to milliseconds, seconds, or otherwise; the number of molecules is fixed at 1 million (1,000,000). After this time $t + 10\Delta t$, we observe the particle position distribution along the x -axis.

¹² This is the principle of electrophoresis, used to separate proteins by electric charge—from the Greek “phórēsis,” meaning to transport, migrate.

¹³ This is quantifiable—called the thermal bath—and equals $K_B T$, the product of Boltzmann’s constant and absolute temperature in Kelvin.

First, a position distribution curve is generated, and more precisely, the cumulative frequency curve is added: what proportion of molecules has reached distance x . What varies in our simulation experiment is the electric field strength E ; temperature remains constant. We shall call $\beta = \frac{qE}{K_B T}$ the ratio of these two forces. It equals zero when no field is applied, and rises to 3 here when the directional force from the field on the protein is three times stronger than the random thermal agitation.

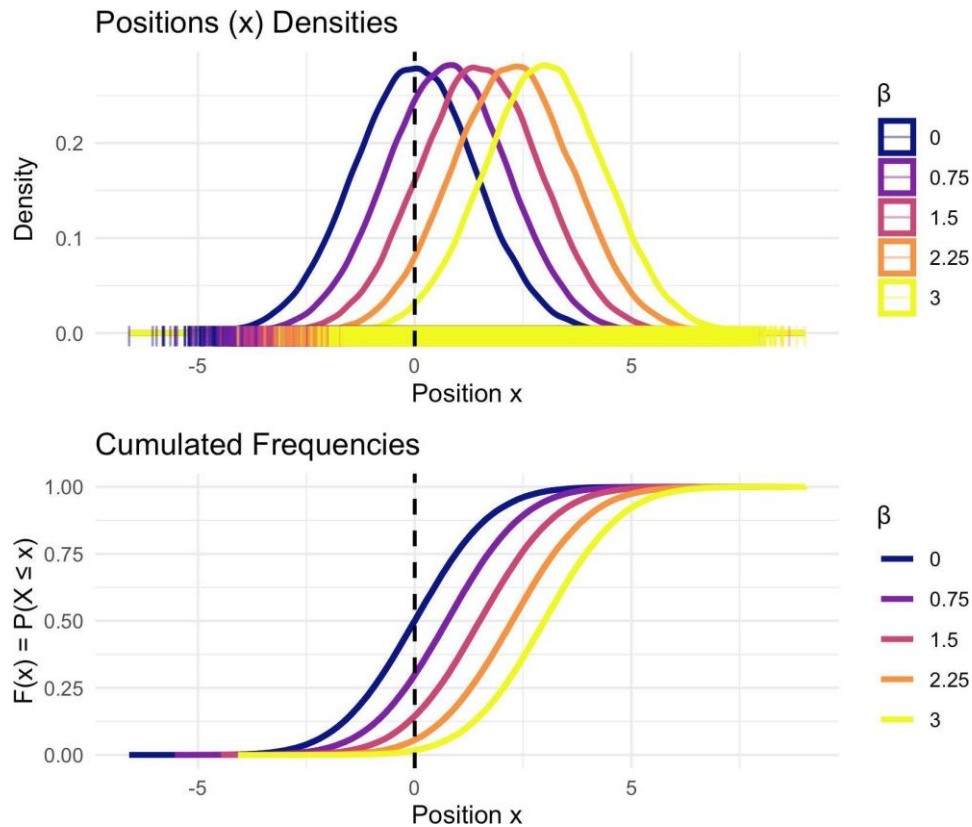


Figure 5. Evolution of skewness as the electric field strength increases

If molecules are subject only to thermal agitation ($\beta = 0$), final positions (blue curves) follow a perfectly symmetric “bell curve” distribution with zero mean: as many molecules migrate left as right. If the electric field is now non-zero ($\beta > 0$), more molecules migrate rightwards under this force: the stronger the field ($\beta \gg 0$), the more the distribution shifts towards positive x -values and becomes asymmetric. The shift of the cumulative frequency curves shows this: just look at the mean position ($F(x) = 0.50$) that half of the molecules have reached. With no field, this equals zero (symmetric displacements on either side of the starting point); for $\beta = 3$, it reaches about $x = 3$, meaning that 500,000 molecules (half) reach a distance of +3 after 10 time steps.

Here, counter-migrating molecules dwindle, acquiring an increasingly random character. These rare trajectories reveal non-linear instabilities. The practical impact of these few “delayed” molecules matters, as they affect the global dynamics. As noted earlier, this technique separates molecules by charge. Yet real physical systems have constrictions—local narrowings in migration channels where the electric field intensifies sharply, altering mean speeds through heightened trapping.

For $\beta > 1$, this trapping halts net flux. Beyond $\beta > 1.5$, convection emerges, spawning chaotic swirls near traps that release molecules not continuously but in bursts—boosting mass transport by up to tenfold: molecules then clearly separate and are detected in packets, raising the technique’s resolution through turbulent (not laminar) flow. Thus, equilibrium conditions end as soon as $\beta > 1.5$, with chaotic convection: turbulence appears.

We see, then, that these rare events alter the mean speed, increase trapping, and paradoxically improve molecule separation. In biomedical applications, these abnormal flows concentrate analytical targets, making analyses more sensitive despite the underlying dynamic complexity. Indeed, charged particles often serve as biomarkers (proteins, cancer exosomes). Normal trajectories ($\beta < 1$) disperse them uniformly in the channel. At $\beta > 1$, selective trapping preferentially holds larger or more polarizable ones at constrictions, whereas smaller ones pass. Chaotic convection ($\beta > 1.5$) then creates concentrated bursts: instead of a diffuse flow, biomarkers arrive in intense peaks at detectors, boosting the analytical signal by a factor of ten.

Thus, these rare events transform passive migration into enriched dynamic separation, enhancing trace cancer detection despite a lower mean flux.

These macroscopic flow changes—selective trapping, concentrated bursts, and signal enhancement—stem directly from the linear non-equilibrium regime initiated by rare negative trajectories at $\beta = 1$. In the linear regime ($\beta < 1$), modelling¹⁴ predicts straight trajectories and uniform, laminar flow without inversions or chaos. At $\beta > 1$, nonlinear trapping breaks this linearity: microscopic fluctuations (negative trajectories) propagate into collective instabilities, creating trapping and convection that are impossible at equilibrium.

Without nonlinearity, no bifurcation to chaos occurs; rare events are the observable precursors of this shift to active dynamics that enrich biomarker separation. Thus, linear modelling fails precisely

¹⁴ Hückel–Smoluchowski.

because it ignores these effects, confirming that macroscopic non-equilibrium arises from microscopic imbalance.

Conclusion

Where have we landed? From the erratic behaviour of simple gas particles, we have drifted towards the history of events that make History—unexpected events weaving the fabric of our daily reality. For living systems, inherently far from equilibrium, bifurcations set the clocks. Every new second, every moment, holds the potential to act as a starting point for time’s arrow, since rare events decisively shape the future, and thus our history [9].

The apparent level of complexity of the analysed subsystems matters little: from atoms (or subatomic particles) to the universe, via galaxy clusters, every system is a system of out-of-equilibrium systems where outsiders hold sway over their destiny. Rare events are not “anecdotal” or mere “news scoops”: they drive the creative forces of our worlds. *What comes into the world to disturb nothing deserves neither regard nor patience* [16].

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Appendix

A new model was designed, based on a 32×32 matrix whose $n = 1024$ cells can host from 0 to N particles. As before, a random—thus non-uniform—initial distribution is set for N particles ($32 \leq N \leq 4096$). This initial distribution then evolves (at constant N) over a few (8) time steps according to a simple rule deliberately mimicking gravity: the probability that a given cell hosts additional particles at any moment is weighted by the square of the number of particles it hosted at the previous instant. This results in faster or slower particle clustering, depending on the initial partition and the particle-to-cell ratio (N/n).

Here, the thermodynamic entropy calculation equations are as follows: for a 32×32 matrix containing N particles distributed non-uniformly, we use Boltzmann's entropy formula with the Stirling approximation.

Stirling approximation for a large number of particles n :

$$\ln(n!) \approx n \ln n - n$$

Total system entropy: entropy S is proportional to the logarithm of the number of micro-states Ω :

$$S = k_B \ln \Omega$$

Where

$$\Omega = \frac{N!}{\prod_{i=1}^{1024} n_i!}$$

with

$$N = \sum_{i=1}^{1024} n_i \text{ total number of particles in the matrix}$$

n_i : number of particles in cell i .

Using the Stirling approximation, entropy becomes:

$$S = N \ln N - N - \sum_{i=1}^{1024} [n_i \ln n_i - n_i]$$

Simplifying (since $N = \sum_{i=1}^{1024} n_i$):

$$S = N \ln \ln N - \sum_{i=1}^{1024} * ni \ln ni$$

High entropy (e.g., uniform distribution) indicates that particles are spread homogeneously (maximum disorder), whereas low entropy (e.g. concentrated distribution) indicates that they are clustered in a few cells (high order). These equations quantify the entropy decrease as particles aggregate, reflecting the growing order in the corresponding system. Figures 4 and 5 show how particle spatial distributions and the resulting entropy evolve over time for $N = 512$ and $N = 4096$, respectively.

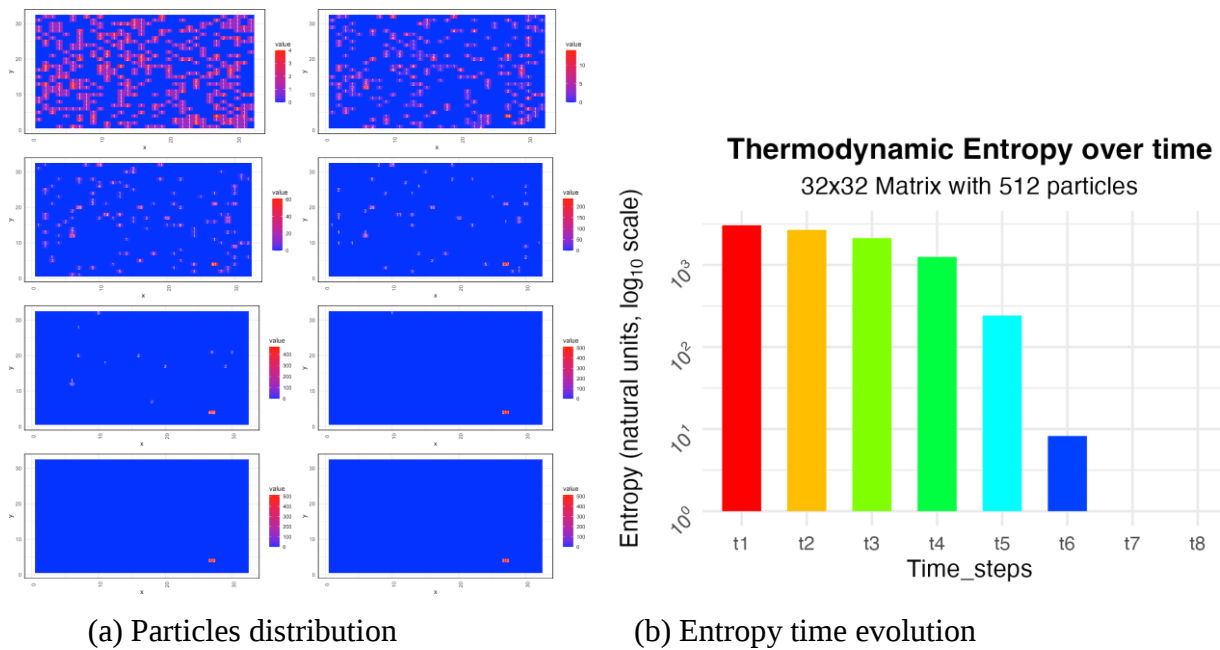


Figure 6. Model $\frac{N}{n} = 0,5$

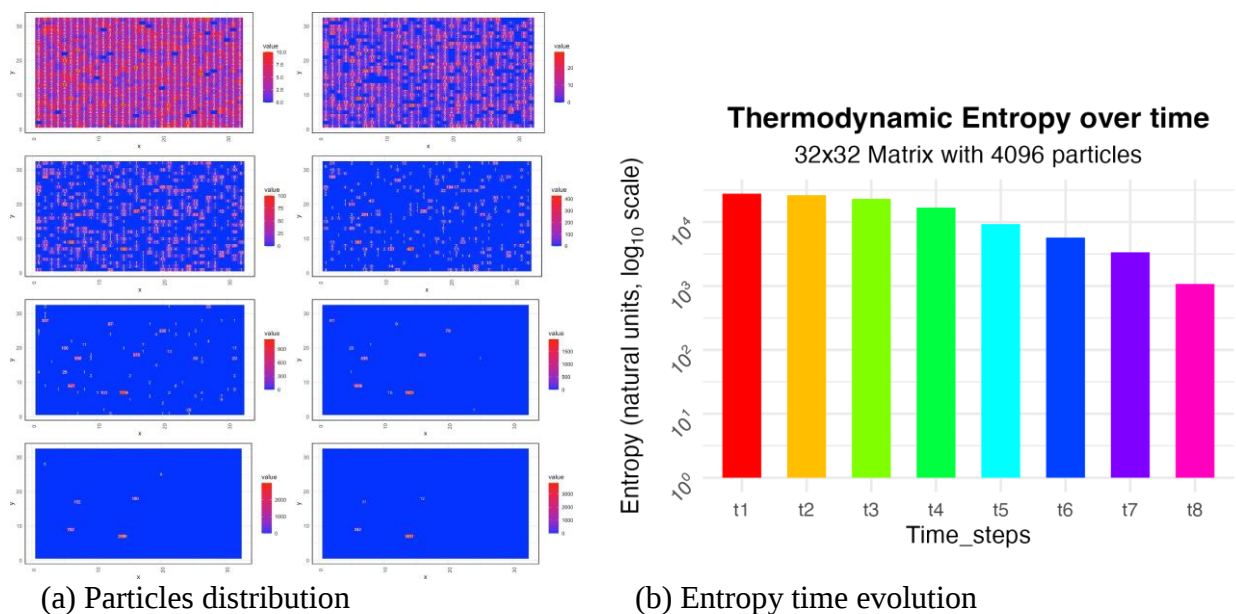


Figure 7. Model $\frac{N}{n} = 4$

Figures 6a and 7a show, over the 8 time steps, how many particles each cell hosts. Figures 6b and 7b present the corresponding entropy evolution over time, on a logarithmic scale. A zero value, as seen at $t = 7$ and $t = 8$ for $N = 512$, indicates that all particles have migrated to a single cell.

We observe that in “crowded” conditions ($N = 4096$), cluster formation is slower and, consequently, entropy decrease is proportionally weaker compared to “sparse” conditions ($N = 512$), where particles quickly cluster into one cell, giving birth to a tiny, rare, low-entropy world. Note that “fast” and “slow” here are purely qualitative, as are the time steps, which do not correspond to any defined temporal span.

Focusing now on the fate of an initially very dense subsample ($N = 4096$) of the universe, we observe that it evolves towards four small worlds whose sizes span three log units (Fig. 8): in practice, the smallest hosts 11 particles, whereas the largest contains nearly 4,000.

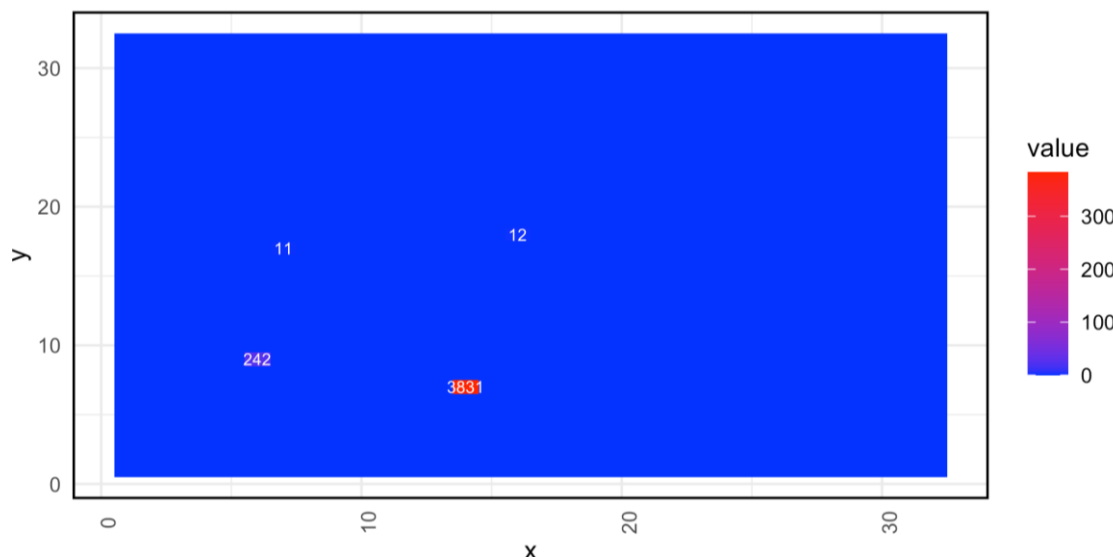


Figure 8. Particles distribution at $t = 8$ for $N = 4096$

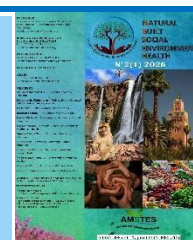
These four “worlds” will likely now evolve differently, as a result of distances and internal particle interactions, which must depend on their respective sizes and mutual separations. One is tempted to see fractal-like properties linking the behaviour of a low-interaction particle with its environment to that of a denser entity—that is to say, composed of more individuals. The crucial point is that interactions—both in number and in nature—between constituent elementary entities truly drive the dynamics and determine the fate of these subsystems.



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Geological and Pedoclimatic Controls on Soil Evolution and Degradation in the Humid Tropical Gabon: Insights from Ferralitic and Coastal Sedimentary Terrains

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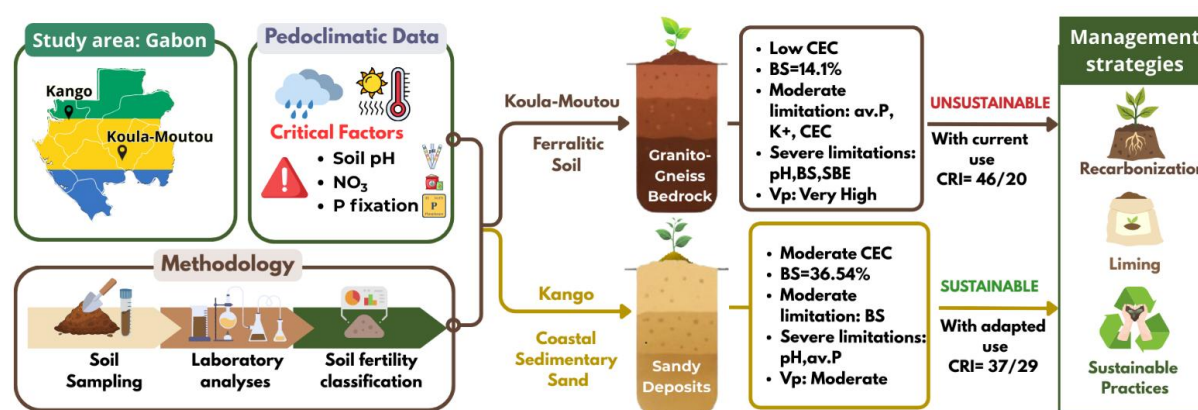
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Graphical abstract

This study examines how lithology and pedoclimate govern soil evolution and degradation in humid tropical Gabon by comparing ferralitic soils developed on ancient granito-gneissic basement at Koula-Moutou to younger coastal sedimentary sands at Kango. Ferralitic soils display extreme acidity (pH < 4.5), low cation exchange capacity, and severe base depletion, reflecting advanced degradation but lower vulnerability, whereas coastal sands retain nutrients more moderately yet remain more prone to nutrient loss, highlighting the need for site-specific management such as liming and organic inputs.



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Abstract: This study investigates how lithology and pedoclimate control soil evolution and degradation across two contrasting systems in humid tropical Gabon. The research compares ferralitic soils formed on ancient granito-gneissic basement at Koula-Moutou to younger coastal sedimentary sands at Kango. Physicochemical analyses revealed divergent trajectories: ferralitic soils exhibited extreme acidity (pH < 4.5), low cation exchange capacity, and severe base depletion, rendering them highly degraded but less vulnerable through persistent weathering. Conversely, coastal sands showed moderate nutrient retention but greater vulnerability to nutrient loss. These findings highlight the fact that lithological inheritance governs fertility dynamics. There is a need for site-specific management, such as liming and organic inputs, to restore Congo Basin soils.

Keywords : Lithological inheritance, Tropical soil degradation, Sustainable soil management.

1. Introduction

Soils link the lithosphere, biosphere, and climate systems. Soils are fundamental in regulating ecosystem processes such as nutrient cycling, water retention, carbon sequestration, and primary productivity [1,2]. Many soils, particularly tropical soils, are considered to be degraded [3], and these functions are increasingly compromised by abiotic and biotic factors, including climate variability, parent material, and anthropogenic activities [4]. Covering approximately 40% of the Earth's terrestrial surface [5,6] and exhibiting heightened sensitivity to degradation, tropical soils are a frontier for global sustainability initiatives [7,8]. Central to the "4 per 1000" Initiative, these soils are critical to promote a transition toward productive, resilient, and climate-smart agriculture rooted in sustainable land and soil resource management [9]. Tropical soils also play a strategic role in advancing several interlinked United Nations Sustainable Development Goals (SDGs), including food security (SDG 2), sustainable land use (SDG 12), climate action (SDG 13), and biodiversity conservation (SDG 15) [10].

In Central Africa, particularly in Gabon, the interplay between complex bedrock geology and high rainfall regimes has produced a striking geopedological mosaic. Soils derived from contrasting parent materials such as highly weathered ferralitic soils formed on the Precambrian crystalline basement and relatively younger coastal sedimentary sands developed on Cretaceous–Tertiary formations exhibit divergent physical and chemical behaviours [11]. These lithological differences influence not only the mineralogical and structural properties of soils but also their fertility potential and degradation pathways under increasing anthropogenic pressure [12].

The functional differentiation of soils reflects their underlying geological heritage and their response to climatic drivers, both critical to landscape evolution and long-term soil sustainability. Fertility is governed by interrelated variables such as texture, organic carbon content, base saturation, and nutrient retention—all shaped by the mineralogy of the parent material, the intensity of weathering, and the input of organic matter associated with vegetation cover [13–16]. Moreover, these parameters influence the vulnerability of soils to degradation, particularly in agricultural landscapes and areas affected by deforestation [17,18].

The study areas, Koula-Moutou in southeastern Gabon and Kango in the western coastal belt, offer contrasting litho-climatic contexts. Koula-Moutou is located in the Chaillu Massif, a mountainous region straddling the border between southern Gabon and the western Republic of the Congo [19]. This massif is a segment of the ancient Archean basement dominated by

tonalites, granodiorites, and mafic intrusions, indicative of a complex polyphase magmatic and metamorphic history [11,20]. Kango, in contrast, is situated within Gabon's coastal sedimentary basin [21], a geomorphic unit formed during the Early Cretaceous rifting of the South Atlantic. This basin records a complex fluviomarine depositional history, comprising stratigraphic sequences such as the Agoula, Madiela, and Sibang formations [10,11]. These formations, dominated by unconsolidated sands, marls, and occasional carbonates, underpin a landscape of inherently fragile, quartz-rich soils [22]. The coastal sedimentary sands extend far beyond Gabon, forming a continuous belt along the Atlantic margins of Central Africa, notably in Cameroon and the Democratic Republic of the Congo [23]. This transboundary distribution emphasizes that they are a shared geopedological resource—and a common vulnerability—within the Congo Basin's coastal fringe [24,25].

These lithostratigraphic contrasts condition the processes of soil formation, mineral weathering, and nutrient availability. By linking soil processes to their geological substrates and climatic regimes, this research contributes to understanding lithology–soil–climate interactions in the context of tropical landscape evolution and long-term ecosystem resilience. This study investigates how lithological inheritance and pedoclimatic conditions influence the development, degradation, and sustainability of tropical soils in these two divergent settings. The aim is to: (1) characterize the physicochemical properties of ferralitic and coastal sedimentary soils across vertical soil profiles; (2) evaluate fertility levels using a structured classification approach based on integrated soil indicators; (3) assess the soil degradation rate (SDR) and vulnerability potential (V_p) through composite indices to quantify susceptibility to nutrient loss and functional decline; and (4) propose site-specific soil management strategies for each geopedological context.

Given the differences in the geological substrate—which govern not only structural development but, more critically, nutrient bioavailability—we hypothesize that coastal sedimentary sands, despite structural fragility, will show higher nutrient responsiveness and more favourable sustainability indices under improved management conditions, whereas ferralitic soils formed on the crystalline basement will exhibit lower resilience. This hypothesis is tested by comparing soil properties across contrasting geopedological contexts in Gabon.

2. Materials and methods

2.1 Study area and sampling design

Field investigations were conducted during two separate campaigns (December 2023 and February 2024) at two ecologically and geologically distinct sites in Gabon: Koula-Moutou, located in the southeast, and Kango, in the western coastal region (Fig. 1a). Both locations are characterized by secondary forest cover but differ in climatic regimes, floristic composition, and underlying lithology.

The Koula-Moutou site (01°14.686' S, 12°54.743' E) lies within the fully humid equatorial zone and receives a mean annual rainfall of ~2,000 mm, with an average temperature of 26 °C. The vegetation comprises mainly dense tropical forests, gradually transitioning into disturbed secondary growth near human settlements. Dominant species include *Aucoumea klaineana* (Okoumé), *Desbordesia insignis* (Alep), *Pachylobus butettneri* (Ozigo), and *Irvingia gabonensis* (bush mango). Montane zones are frequently colonized by heliophilous pioneers such as *Musanga cecropioides* (parasol tree) and *Aucoumea klaineana*.

In contrast, Kango (00°06.977' N, 10°11.264' E) experiences a transitional equatorial climate, with a similar mean temperature but higher rainfall (~2,566 mm/year). The site has been historically influenced by anthropogenic activity, particularly selective logging and settlement expansion, resulting in floristic compositions dominated by species similar to those of Koula-Moutou but in varied successional stages.

Geologically, Koula-Moutou is part of the Chaillu Massif, underlain by Archean-aged granitoid complexes (e.g., tonalites, granodiorites, and monzogranites) and mafic intrusions, indicative of a polyphase magmatic evolution within the Congo Craton [11,26,27]. This lithostructural setting has promoted the formation of deeply weathered ferralitic soils. In contrast, Kango lies within the coastal sedimentary basin, an Atlantic margin basin formed during the Early Cretaceous, characterized by interbedded continental, lacustrine, and marine formations. The dominant surficial material here is coastal alluvium and sandy sediments, derived from the Agoula, Madiela, Cocobeach, and Sibang sequences [10,27] (Fig. 1b–c).

At each site, composite soil samples were collected at three depth intervals (0–10 cm, 10–20 cm, 20–30 cm), with three replicates per depth, following a randomized spatial layout. A total of 22 soil profiles were surveyed in Kango and 18 in Koula-Moutou, resulting in 120 individual samples from 40 profiles. All samples were air-dried, sieved (2 mm), and stored for physicochemical analysis.

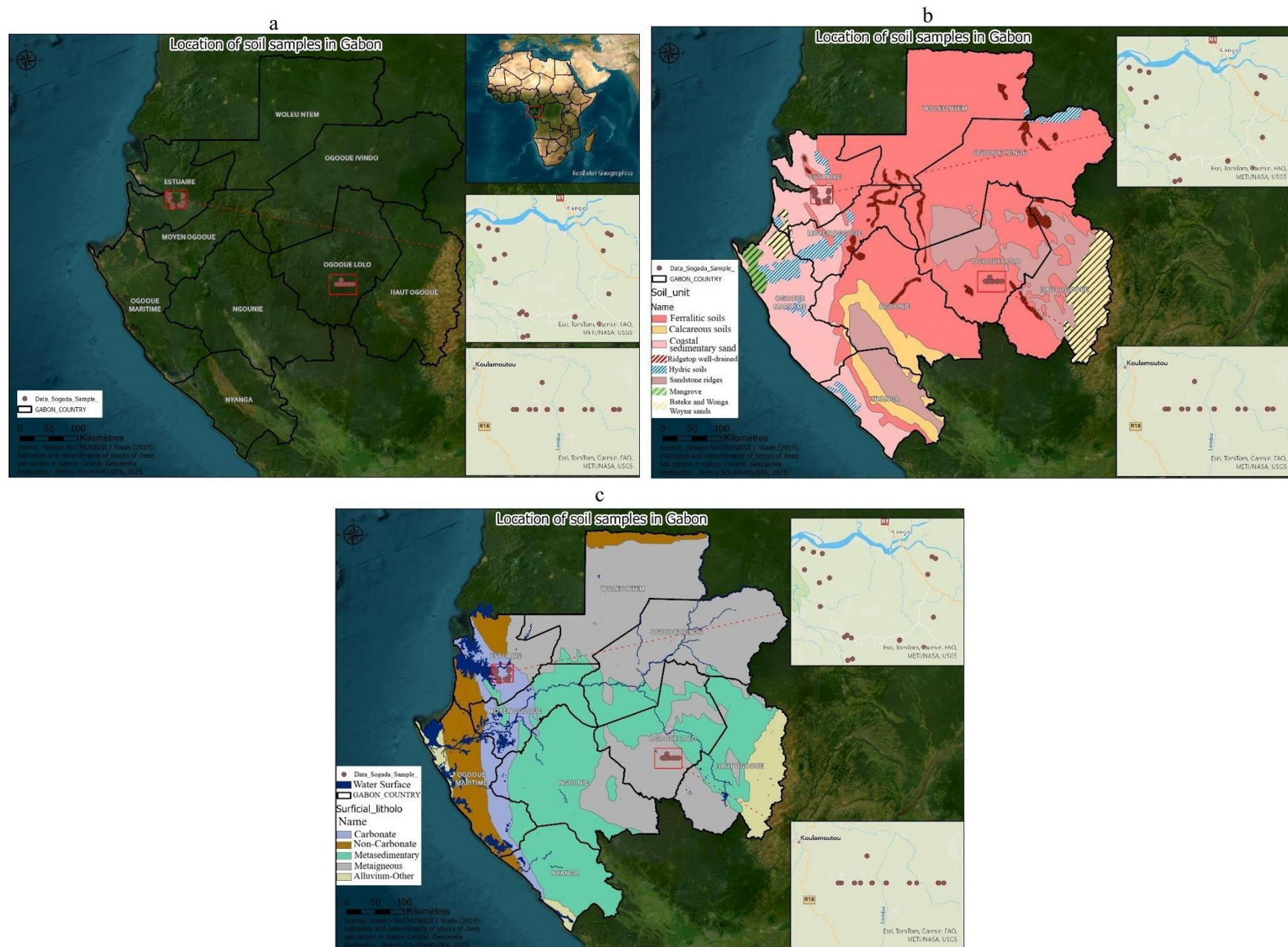


Figure 1. Sampling sites maps showing: (a) vegetation cover, (b) lithology, and (c) soil units. Maps adapted from Wade *et al.* [27]

2.2 Laboratory analyses of soil Properties

Soil pH was measured in a 1:2.5 soil-to-water suspension using a calibrated pH meter. Soil organic carbon (SOC) was quantified using the Walkley-Black dichromate oxidation method [28]. Total nitrogen (TN) was determined via Kjeldahl digestion [29]. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined spectrophotometrically, following [30].

Available phosphorus was extracted using 0.5 M NaHCO_3 and analysed colorimetrically [31]. Exchangeable base cations (Ca^{2+} , Mg^{2+} , K^+ and Na^+) were extracted using a 1 M ammonium acetate solution (pH 7.0). Ca^{2+} and Mg^{2+} were quantified using EDTA titration, whereas K^+ and Na^+ were determined via flame photometry [32]. The cation exchange capacity (CEC) was determined using the 1 M ammonium acetate method (pH 7.0) [33].

Soil particle size distribution was assessed using the pipette method. Bulk density (Bd) was estimated using pedotransfer functions [34]. Soil water content (WC), total porosity (TP), and air porosity (AP) were calculated following [35]. Soil structural stability was evaluated using the Index of Soil Structural Stability (ISS) [36]:

$$ISS (\%) = \frac{1.724 \times OC}{Silt + Clay} \times 100$$

Where OC represents the organic carbon content. Interpretation thresholds were defined as follows: $ISS > 9\%$: high structural stability; 7–9%: moderate stability, low risk; 5–7%: high degradation risk; $\leq 5\%$: structurally degraded soils [36].

2.3 Soil fertility classification and degradation assessment

To assess current fertility status, soils were classified using the framework of [Quemada and Cabrera [37], refined by Nguemezi et al. [38]. Soils were assigned to one of four fertility classes based on the severity and number of physicochemical limitations: Class I (no or minimal limitations), Class II (up to three moderate limitations with low severity), Class III (more than three moderate limitations and at least one severe limitation), Class IV (multiple severe limitations).

The following parameters were included in the assessment: soil texture, SOC, TN, C/N ratio, available P, exchangeable bases (Ca^{2+} , Mg^{2+} , K^+ , Na^+), base saturation (BS), CEC, and pH.

Degradation dynamics were evaluated using two metrics: soil degradation rate (SDR) and vulnerability potential (Vp). SDR scores ranged from 1 (none) to 5 (extreme degradation), whereas Vp scores ranged from 5 (very low vulnerability) to 1 (very high vulnerability).

The Cumulative Rating Index (CRI) (Table 1) was derived by summing the SDR and Vp weighting values, providing an integrated proxy for soil quality and functional stability [39].

Table 1. Interpretation of Cumulative Rating Index

	Cumulative Rating Index	
	SDR	SVP
Sustainability		
Highly Sustainable	<20	>40
Sustainable	20-30	35-40
Sustainable with high input	30-35	30-35
Sustainable with another land use	35-40	20-30
Unsustainable	>40	<20

2.4 Statistical analyses

All data were tested for normality using the Shapiro–Wilk test. When the assumptions of normality were met, one-way ANOVA was applied; in other cases, non-parametric Kruskal–Wallis tests were employed. Post hoc comparisons were performed when required. Relationships among soil properties were explored using Pearson’s correlation and linear regression analyses. All statistical analyses were conducted at a 95% confidence level ($\alpha = 0.05$) using Python (Spyder IDE).

3. Results

3.1 Soil physical properties and profile structure

The particle size distribution and structural properties of soils varied significantly between the two geologically distinct sites (Table 2; Fig. 2). At Kango, coastal sedimentary sands exhibited a loamy texture, whereas Koula-Moutou’s ferrallitic soils were classified as sandy clay. In both soils, sand was the dominant fraction; the overall distribution of sand, silt, and clay showed contrasting depth trends (Fig. 2a–c).

Clay content increased with depth at both sites, reaching higher proportions in Koula-Moutou, where it constituted the second most abundant fraction. By contrast, silt was more abundant in surface horizons and declined with depth, particularly in the Kango profiles. These opposing trends were reflected in the silt/clay ratio, which was significantly higher in the coastal sedimentary sands (Kango) than in the ferrallitic soils (Koula-Moutou; $p < 0.05$). Quantitatively, the arable layer (0–30 cm) at Koula-Moutou contained 85% more clay and 65% more silt than its counterpart in Kango ($p = 1.5 \times 10^{-12}$).

Bulk density (Bd) was significantly lower across all depths in Kango soils compared to Koula-Moutou ($p = 2.0 \times 10^{-9}$, Table S1), consistent with a looser, coarser sediment structure (Fig. 2d). Conversely, the Index of Soil Structural Stability (ISS) was significantly higher in the surface horizons of the ferrallitic soils ($p = 1.2 \times 10^{-3}$), but declined with depth at both sites (Fig. 3a). Although water content (WC) and air porosity (AP) did not differ significantly between sites ($p > 0.05$), total porosity (TP) was notably greater in Kango soils ($p = 2.0 \times 10^{-9}$), especially in the upper 10 cm, consistent with their lower compaction and higher sand fraction (Fig. 3b–d).

Table 2. Mean soil physical properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	<i>n</i>	Depth(cm)	Clay (%)	Sand (%)	Silt (%)	Silt:Clay	Texture	Bd (g/cm)	ISS (%)	WC (%)	AP (%)	TP (%)
Coastal sedimentary sands	21	0-30	14.22 ± 6.44 ^a	52.15 ± 8.58 ^a	33.63 ± 7.69 ^a	2.84 ± 1.45 ^a	Loam	1.44 ± 0.01 ^a	9.03 ± 3.69 ^a	22.19 ± 3.16 ^a	21.89 ± 3.01 ^a	45.61 ± 0.40 ^a
Ferralitic soils	18	0-30	26.29 ± 8.36 ^b	61.90 ± 8.50 ^b	11.81 ± 4.79 ^b	0.59 ± 0.54 ^b	Sandy clay loam	1.48 ± 0.02 ^b	14.46 ± 7.53 ^b	14.22 ± 4.36 ^a	21.85 ± 4.29 ^a	44.06 ± 0.79 ^b

n : Number of samples ; Bd: Bulk density ; ISS : Soil aggregate stability index ; WC : Water content ; AP : Air porosity ; TP : Total porosity, Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.

Table 3. Mean soil chemical properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	<i>n</i>	Depth (cm)	pH	SOM (%)	SOC (%)	TN (%)	C/N	NO_3^- (ppm)	NH_4^+ (ppm)
Coastal sedimentary sands	21	0-30	4.61 ± 0.23 ^a	3.84 ± 0.98 ^a	2.22 ± 0.57 ^a	0.24 ± 0.6 ^a	9.57 ± 1.84 ^a	16.12 ± 5.76 ^a	32.63 ± 9.85 ^a
Ferralitic soils	18	0-30	3.76 ± 0.42 ^b	4.64 ± 1.28 ^b	2.69 ± 0.74 ^b	0.23 ± 0.08 ^a	11.87 ± 1.61 ^b	14.58 ± 4.75 ^a	30.32 ± 11.40 ^a

Table 4. Mean soil fertility properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	<i>n</i>	Depth (cm)	av.P (ppm)	Ca ²⁺ (cmol/kg)	Mg ²⁺ (cmol/kg)	K ⁺ (cmol/kg)	Na ⁺ (cmol/kg)	CEC (cmol/kg)	SBE (cmol/kg)
Coastal sedimentary sands	21	0-30	4.74 ± 1.63 ^a	3.80 ± 1.53 ^a	1.34 ± 0.59 ^a	0.32 ± 0.1 ^a	0.07 ± 0.05 ^a	16.73 ± 4.30 ^a	6.10 ± 3.17 ^a
Ferralitic soils	18	0-30	8.17 ± 2.51 ^b	0.64 ± 0.40 ^b	0.24 ± 0.16 ^b	0.11 ± 0.04 ^b	0.04 ± 0.04 ^a	8.73 ± 3.12 ^b	1.05 ± 0.57 ^b

n: Number of samples ; av.P : Available phosphorus; Ca²⁺ : Exchangeable calcium; Mg²⁺ : Exchangeable magnesium; K⁺ : Exchangeable potassium; Na⁺ : Exchangeable sodium; CEC: Cationic exchangeable capacity; SBE : Sum exchangeable bases. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.

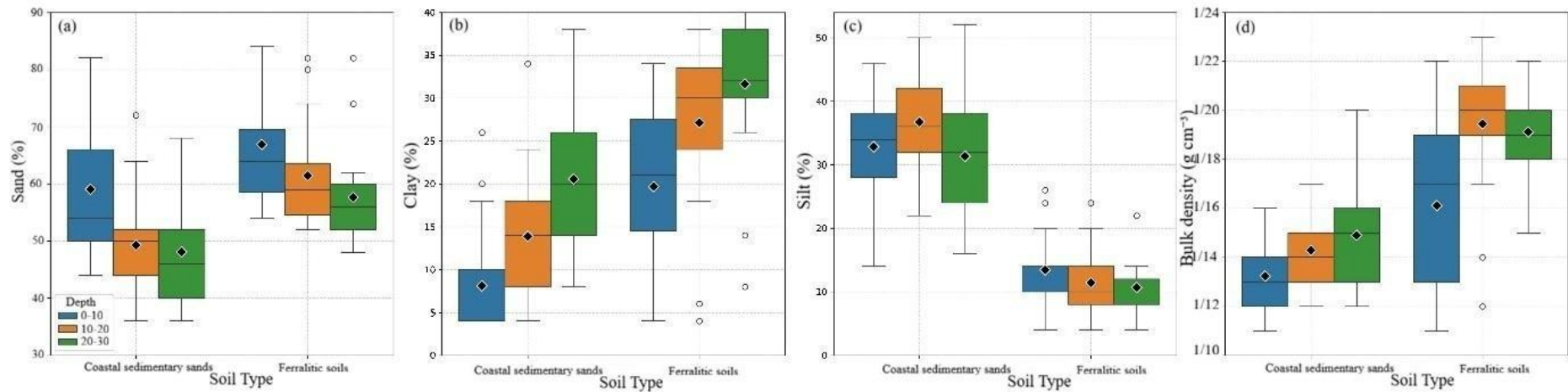


Figure 2. Distribution of physical soil quality indicators by soil type: (a) Sand, (b) Clay, (c) Silt, and (d) Texture. Box plots show the interquartile range (25th–75th percentiles), with the median (solid line), mean (diamond), and outliers (dots).

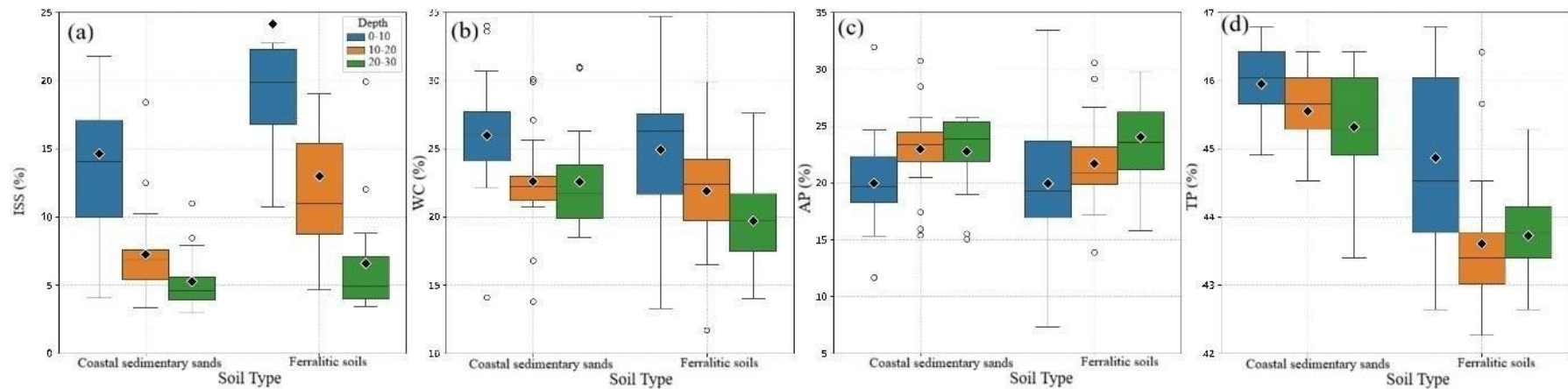


Figure 3. Distribution of structural and porosity-related indicators: (a) ISS (Soil Structural Stability Index), (b) WC (Water Content), (c) AP (Air Porosity), and (d) TP (Total Porosity). Box plot elements as in Figure 2.

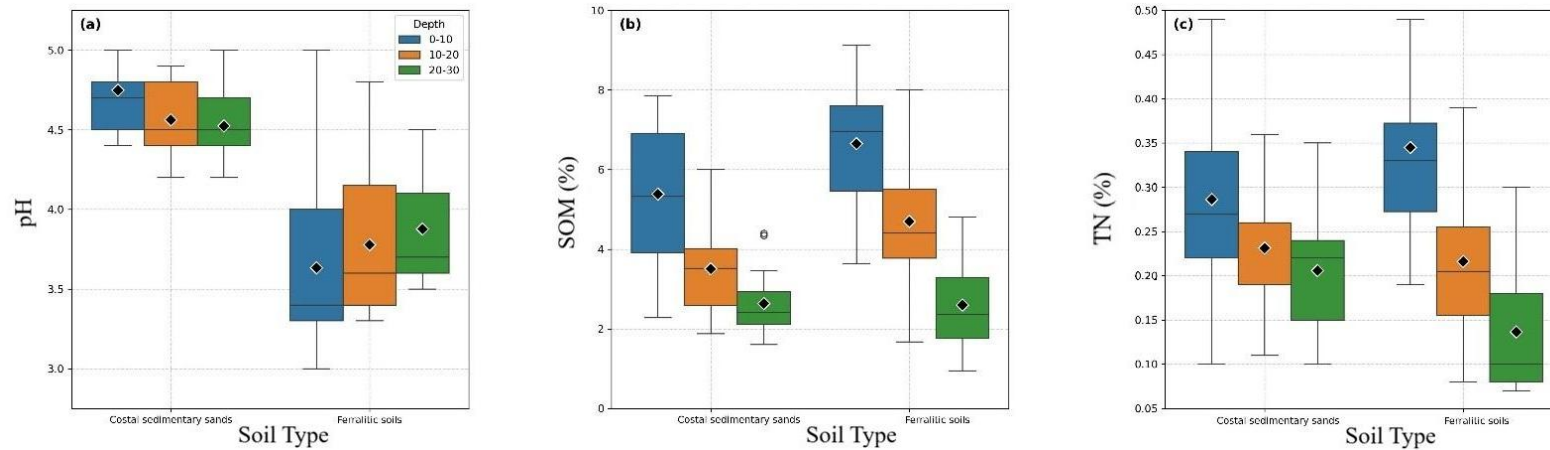


Figure 4. Distribution of chemical soil indicators: (a) pH, (b) SOM (Soil Organic Matter), (c) TN (Total Nitrogen). Box plot elements as in Figure 2.

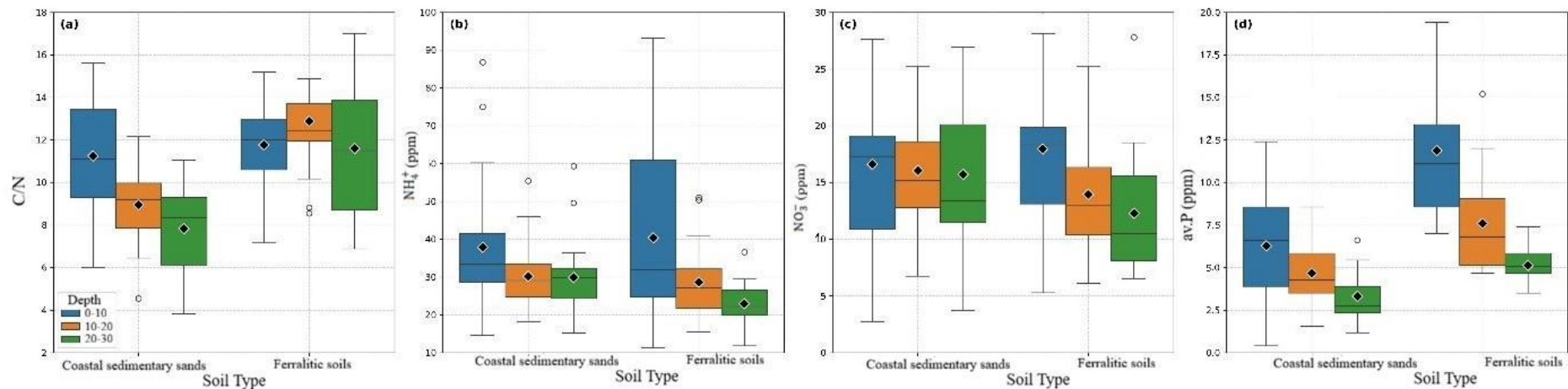


Figure 5. Distribution of nitrogen and phosphorus indicators: (a) C/N ratio, (b) Ammonium (NH_4^+), (c) Nitrate (NO_3^-), (d) av.P (Available Phosphorus). Box plot elements as in Fig. 2.

3.2 Chemical characteristics of soil horizons

The chemical fertility of the soils showed sharp contrasts between the two lithologically distinct systems (Table 3; Fig. 4). Mean pH values were significantly higher in the coastal sedimentary sands (+0.85 unit, $p = 1.4 \times 10^{-6}$), with declining acidity in surface layers and increased acidification with depth in Kango. By contrast, Koula-Moutou soils exhibited an upward trend in pH with depth (Fig. 4a).

Soil organic matter (SOM) content was significantly enriched in the arable layers of ferralitic soils (+21%, $p < 0.05$), although both sites displayed a general decrease in SOM with depth (Fig. 4b, Table S2). Total nitrogen (TN) levels were similar across soil types with no significant difference ($p > 0.05$); a steeper vertical decline in TN was observed in Koula-Moutou (−60%) than in Kango (−38%; Fig. 4c).

C/N ratios were significantly higher in Kango soils, indicative of slower organic matter mineralization ($p = 2.5 \times 10^{-5}$, Fig. 5a). This ratio declined with depth in Kango but remained relatively stable in Koula-Moutou. In coastal sands, these differences were not statistically significant. Although both nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were slightly elevated in coastal sedimentary sands (+8% and +11%, respectively), these differences were not statistically significant (Fig. 5b–c).

3.3 Nutrient distribution and soil fertility indices

The spatial distribution of macronutrients and cation exchange capacity (CEC) revealed divergent nutrient retention strategies (Table 4, Figs. 5d, 6, and 7).

Available phosphorus (available P) was significantly higher in the ferralitic soils (+72%, $p = 1.3 \times 10^{-5}$, Table S3), despite the overall nutrient impoverishment typical of highly weathered profiles. In both soils, available P declined with increasing depth (Fig. 5d).

Exchangeable base cations followed a consistent hierarchy: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$. Their absolute concentrations were markedly higher in Kango soils: Ca^{2+} (+484%), Mg^{2+} (+452%), K^+ (+179%) and Na^+ (+68%). These differences were reflected in the significantly higher CEC (+92%, $p = 1.1 \times 10^{-7}$) and sum of exchangeable base cations (SEB) in Kango soils (+475%, Table S3). CEC remained relatively stable with depth in Kango, whereas in Koula-Moutou it declined sharply (−57.5%). Notably, the exchangeable base cations in Kango decreased with depth ($p = 1.4 \times 10^{-7}$), whereas Koula-Moutou soils showed minimal depth variation (Fig. 7a–b). Base saturation (BS) was also higher in coastal sedimentary sands ($p = 8.5 \times 10^{-6}$) and declined moderately with depth at Kango (−36%), but remained nearly unchanged in Koula-Moutou (Fig. 7c, Table S4).

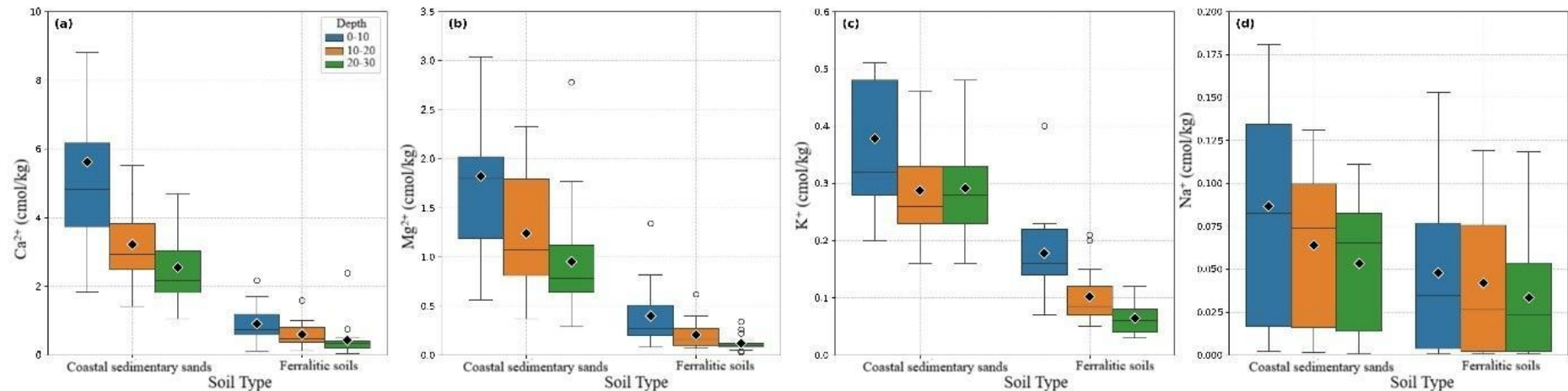


Figure 6. Distribution of exchangeable base cations: (a) Ca^{2+} (Calcium), (b) Mg^{2+} (Magnesium), (c) K^+ (Potassium), (d) Na^+ (Sodium). Box plot elements as in Figure 2.

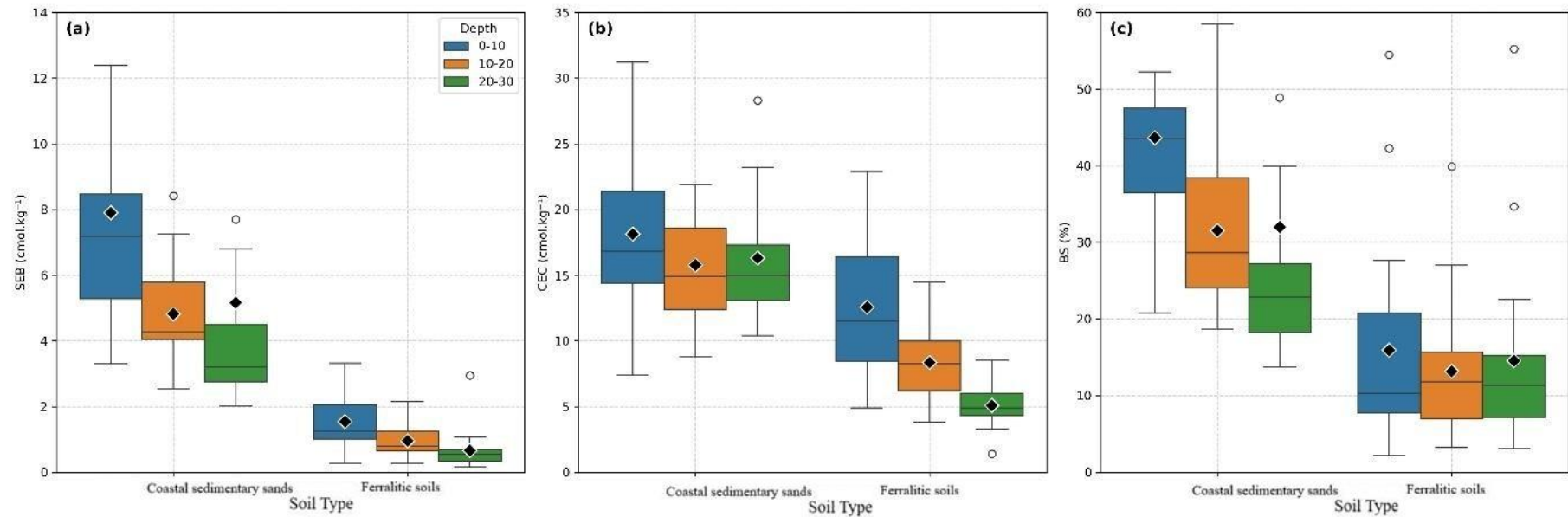


Figure 7. Distribution of nutrient capacity indicators: (a) SEB (Sum of Exchangeable Bases), (b) CEC (Cation Exchange Capacity), (c) BS (Base Saturation). Box plot elements as in Figure 2.

The $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio was stable in Kango but increased slightly with depth in Koula-Moutou. The $\text{Mg}^{2+}/\text{K}^+$ ratio, in contrast, showed a significant decline in Kango and remained unchanged in Koula-Moutou. Differences in $\text{Mg}^{2+}/\text{K}^+$ ratios between the two soil types were highly significant ($p = 9.7 \times 10^{-6}$, Table 5).

Table 5. Mean soil chemical fertility indicators (BS, $\text{Ca}^{2+}/\text{Mg}^{2+}$, $\text{Mg}^{2+}/\text{K}^+$) for the studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	<i>n</i>	BS (%)	$\text{Ca}^{2+}/\text{Mg}^{2+}$	$\text{Mg}^{2+}/\text{K}^+$
Coastal sedimentary sands	21	36.54 ± 17.09^a	2.96 ± 0.77^a	4.18 ± 1.16^a
Ferralitic soils	18	14.15 ± 11.17^b	2.83 ± 0.77^a	2.11 ± 1.03^b

n: Number of samples; BS: Base saturation; $\text{Ca}^{2+}/\text{Mg}^{2+}$: Calcium-to-Magnesium ratio; $\text{Mg}^{2+}/\text{K}^+$: Magnesium –to–Potassium ratio. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey’s multiple comparison test at the 5% significance level.

3.2 Multivariate structure of soil properties

The Pearson correlation matrix (Fig. 8) revealed key functional linkages among soil indicators. Clay content was negatively correlated with nutrient pools (exchangeable cations, NO_3^-), CEC, pH, and TP, but positively correlated with Bd ($p < 0.0001$). Silt content was positively associated with TP, C/N, and CEC. Sand fraction exhibited positive correlations with ISS and Bd.

Soil pH emerged as a central integrator, positively correlated with exchangeable nutrients, CEC, and SEB, but negatively with SOM and available P. TP exhibited strong correlations with multiple fertility indicators, highlighting its diagnostic value for soil quality.

Principal Component Analysis (PCA) explained 94.03% of the total variance (Fig. 9a; Table 6). The first component (PC1, 59.12%) was dominated by variables indicative of parent material influence (texture, Bd, CEC, exchangeable bases).

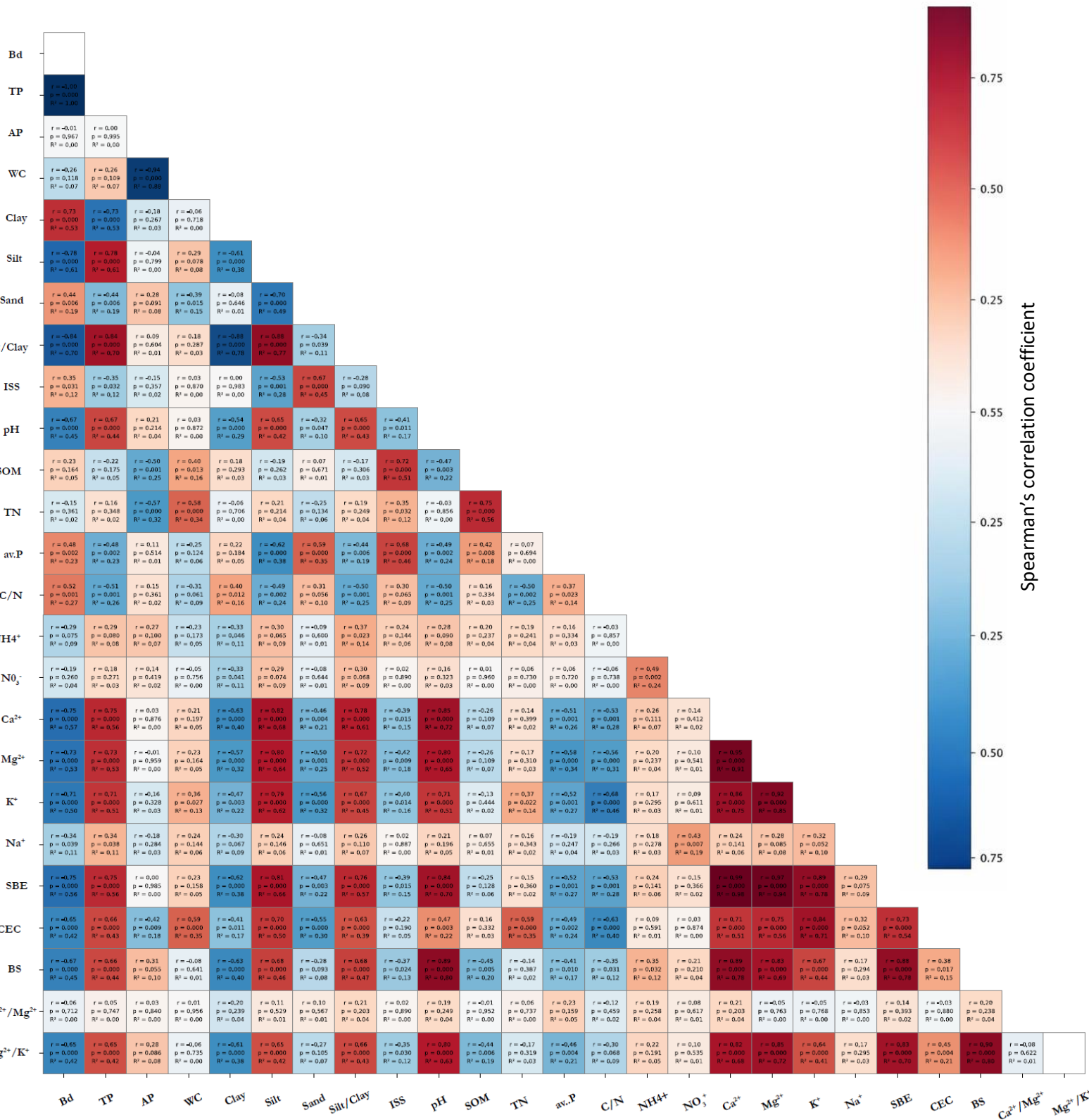


Table 6. Correlation between principal components (PC) and soil quality indicators of the studied soil

SQI	PC1	PC2	p-value
Bd	-0.97	-0.15	0.00005
TP	0.97	-0.15	0.00005
AP	-0.64	-0.75	0.03349
WC	0.87	0.49	0.00521
Clay	-0.98	-0.04	0.00001
Sand	-0.16	0.95	0.00030
Silt	0.82	-0.54	0.01209
ISS	0.22-	0.97	0.00005
pH	0.76	-0.63	0.02900
SOM	0.37	0.92	0.00109
TN	0.60	0.76	0.02748
C/N	-0.41	0.67	> 0.05
NH_4^+	0.66	0.73	0.03967
NO_3^-	0.78	0.49	0.02360
av.P	-0.05	0.99	0.00000
Ca^{2+}	0.91	-0.24	0.00146
Mg^{2+}	0.94	-0.26	0.00052
K^+	0.97	-0.21	0.00004
Na^+	0.96	-0.02	0.00013
CEC	0.97	-0.11	0.00005
BS	0.91	-0.37	0.00195
Eigenvalue	12.24	7.33	
Variance (%)	59.12	34.91	
Cumulative (%)	59.12	94.03	

SQI : Soil quality indicators ; Bd: Bulk density ; ISS : Soil aggregate stability index ; WC : Water content ; AP : Air porosity ; TP : Total porosity ; SOM: Soil organic matter ; TN : Total nitrogen ; C/N : Carbon-to-nitrogen ratio ; NO_3^- : Nitrate ; NH_4^+ : Ammonium ; av.P : Available phosphorus; Ca^{2+} : Exchangeable calcium; Mg^{2+} : Exchangeable magnesium; K^+ : Exchangeable potassium; Na^+ : Exchangeable sodium; CEC: Cationic exchangeable capacity; BS : Base saturation

The second component (PC2, 34.91%) concerned fertility attributes (SOM, TN, ISS, NH_4^+ , available P). PC1 was closely associated with coastal sediments (Kango), whereas PC2 differentiated ferrallitic soils (Koula-Moutou), reflecting divergent pedogenetic and nutrient retention pathways (Fig. 9b).

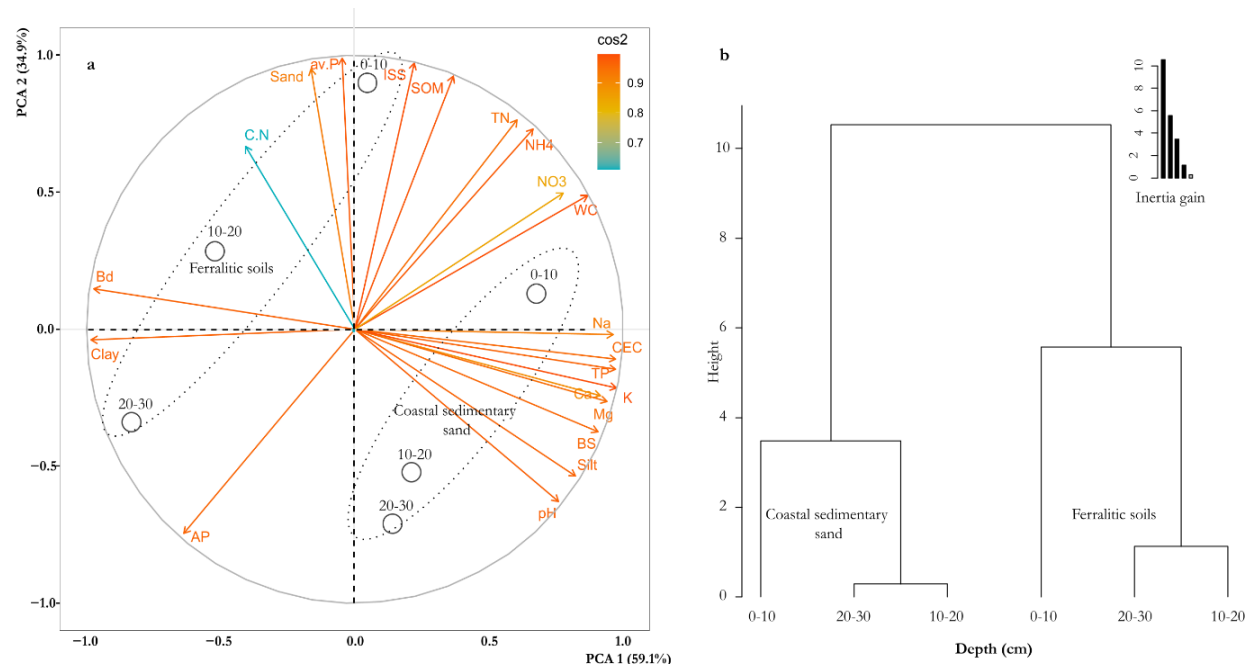


Figure 9. Principal Component Analysis (PCA) of soil quality indicators: **(a)** PCA biplot, **(b)** Hierarchical clustering of soil types based on PCA results.

3.5 Soil fertility classification and degradation assessment

Using the fertility classification, both soils were assigned to Fertility Class IV, indicating severe to moderate limitations. For Kango soils, constraints included low pH and available P, as well as moderate BS deficiency. In Koula-Moutou, limitations were linked to low K^+ and CEC, together with severe deficits in BS, SEB, and pH.

Table 7. Soil types fertility assessment: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site).

SQI	Coastal sedimentary sands	Ferralitic soils
SOM (%)	I	I
TN (%)	I	I
av.P (ppm)	IV	III
K^+ (cmol/kg)	II	III
SEB (cmol/kg)	II	IV
BS (%)	III	IV
CEC (cmol/kg)	II	III
pH	IV	IV
ISS (%)	I	I
Fertility level	Poor	Poor
Limiting factors	pH, av.P, BS,	pH, SEB, BS, CEC, K^+ and av.P
Class	IV	IV

SQI : Soil quality indicators ; SOM: Soil organic matter ; TN : Total nitrogen ; av.P : Available phosphorus; K^+ : Exchangeable potassium; SEB : Sum of exchangeable bases; BS : Base saturation ; CEC: Cationic exchangeable capacity

Degradation metrics further clarified these differences (Table 7). For both soil types, SDR/Vp scores indicated high degradation susceptibility in pH and TN (scores of 5/1), moderate degradation in Bd (3/3), and varying patterns in base cations. Ferralitic soils consistently scored high SDR (5) and low Vp (1) for all cations, whereas coastal sands displayed more moderate degradation potential (e.g., Ca^{2+} : 4/2; Mg^{2+} : 3/3).

The Cumulative Rating Index (CRI) revealed contrasting degradation dynamics: ferralitic soils exhibited higher degradation rates but lower vulnerability (CRI: 46/20), whereas coastal sands showed a lower degradation profile but higher susceptibility (CRI: 37/29). These findings underscore the distinct geopedological constraints and resilience characteristics embedded in each soil system (Table 8).

Table 8. Assessment of soil degradation rate and vulnerability potential of selected soil quality indicators in the soil types.

SQI	Coastal sedimentary sands		Ferralitic soils	
	SDR	Vp	SDR	Vp
Texture	1	5	2	4
Bd	3	3	3	3
pH	5	1	5	1
SOC	3	3	3	3
TN	5	1	5	1
BS	4	2	5	1
av.P	4	2	3	3
CEC	3	3	4	2
Ca^{2+}	4	2	5	1
Mg^{2+}	3	3	5	1
K^{+}	3	3	5	1
CRI	38	28	45	21

SQI : Soil quality indicators ; SDR : Soil degradation rate ; Vp : Vulnerability potential ; SOC: Soil organic carbon ; TN : Total nitrogen ; BS : Base saturation ; av.P : Available phosphorus; CEC: Cationic exchangeable capacity ; Ca^{2+} : Exchangeable calcium; Mg^{2+} : Exchangeable magnesium; K^{+} : Exchangeable potassium; Na^{+} : Exchangeable sodium ; CRI : Cumulative Rating Index.

4. Discussion

4.1 Lithological control on soil texture, structure, and physical properties

The contrasting physical properties observed between Kango and Koula-Moutou reflect the foundational influence of their parent material, which governs particle size distribution, structural organization, and hydrological behaviour. At both sites, sand dominates the arable layer (>50%), a hallmark of regional pedogenic patterns across Gabon [23,27]. The ferralitic soils at Koula-Moutou, derived from intensely weathered metaigneous substrates of the Chaillu

Massif, exhibit a markedly higher clay content than the coastal sedimentary sands at Kango, which developed on less altered marls and sandstones [10,11]. This contrast underscores the role of geological inheritance in shaping long-term pedogenesis.

The lower silt/clay ratio in Koula-Moutou soils signals an advanced weathering stage, characteristic of mature tropical Ferralsols. This process is marked by silt eluviation and in situ clay accumulation [40,41]. This interpretation is quantitatively supported by a strong negative correlation between silt and clay ($r = -0.61$, $p < 0.0001$), pointing to systematic mineralogical differentiation over time [42].

Texture and mineralogy also shaped the mechanical integrity of the soil matrix. The higher ISS in the finer-textured ferrallitic profiles suggests enhanced aggregation and resistance to erosive forces, a crucial asset under intense tropical rainfall [36]. Conversely, coastal sands, though more porous and aerated, exhibited lower ISS and Bd, rendering them more vulnerable to physical breakdown, rapid infiltration, and nutrient leaching—especially under unsustainable land use [42].

Lithological control extended to Bd and TP. Koula-Moutou soils exhibited higher compaction and reduced moisture retention, probably associated with clay-dominated matrices and sesquioxide enrichment [41–43]. The observed perfect inverse relationship between Bd and TP ($r = -1.00$, $R^2 = 1.00$, $p < 0.0001$; Fig. 8) underscores the tight coupling between mineralogical composition, structural properties, and hydrological functioning in tropical soils. These insights carry critical implications for erosion risk, water availability, and land-use suitability, reinforcing the need for pedologically informed management strategies in contrasting tropical ecosystems [44].

4.2 Lithological control on soil acidity, nitrogen dynamics, and organic matter stabilization

The chemical differentiation of soils across the study sites strongly reflected their lithological heritage (Table 3). Both soil types exhibited acidic conditions, but the contrast in intensity was striking: ferrallitic soils at Koula-Moutou displayed extreme acidity ($\text{pH} < 4.5$), a hallmark of deep tropical weathering and prolonged leaching. By contrast, the coastal sedimentary sands at Kango maintained very strong acidity ($4.5 < \text{pH} < 5.5$), consistent with their carbonate-depleted, quartz-dominated provenance [27,45]. These pH regimes not only differentiated

geochemical signatures but also exerted a profound influence on nutrient dynamics and organic matter stabilization [46].

Although SOM levels were significantly higher in the ferralitic soils (+21%), the mechanisms underlying SOM persistence differed between the two sites. At Koula-Moutou, where extreme acidity and high Fe/Al sesquioxides co-occur, SOM stabilization is probably mediated by organo-mineral associations, including ligand exchange and surface complexation on oxide mineral surfaces [47]. This interpretation is reinforced by negative pH–SOM ($r = -0.47$, $R^2 = 0.22$, $p = 0.003$) and pH–clay ($r = -0.54$, $R^2 = 0.29$, $p < 0.0001$; Fig. 8) correlations, suggesting that mineral surface chemistry plays a critical role in organic carbon retention under low-pH, high-clay conditions [48].

Yet, the acidic environment also imposes biochemical constraints, particularly on nitrogen cycling. Microbial nitrification is suppressed under low pH, leading to an accumulation of ammonium (NH_4^+) at the expense of nitrate (NO_3^-) —a pattern observed at both sites but especially pronounced in the ferralitic profiles, where acidity and clay jointly inhibit nitrifier populations [49]. By contrast, the coarser texture and better-aerated conditions of the coastal sands appear to support greater NO_3^- availability, hinting at more efficient SOM mineralization and improved microbial activity despite their lower SOM stocks [50].

Together, these findings illustrate how geology-mediated pH regimes modulate nutrient retention, microbial function, and organic matter dynamics, emphasizing the need for context-sensitive soil fertility interventions. In ferralitic soils, efforts must focus on pH correction and enhancement of bioavailable nitrogen, whereas in coastal sands, maintaining organic inputs and buffering against acidification will sustain nutrient cycling and productivity [51].

4.3 Phosphorus limitation and nutrient retention patterns

Although phosphorus (P) concentrations appeared elevated in the ferralitic soils of Koula-Moutou relative to the coastal sedimentary sands of Kango, this trend reflects analytical bias rather than agronomic reality. The commonly employed Olsen method—designed for neutral to alkaline soils—tends to overestimate P availability under acidic conditions, a flaw particularly pronounced in tropical weathered soils dominated by Fe and Al sesquioxides [52]. These sesquioxides exhibit strong sorptive affinities for phosphate, effectively sequestering it into insoluble forms that are quantified chemically but inaccessible biologically [53].

In this study, the extremely low pH values recorded in the ferrallitic soils ($\text{pH} < 4.5$; Fig. 5d) further exacerbate P sorption, masking an underlying phosphorus deficiency beneath seemingly adequate concentrations. Hence, the observed P enrichment in ferrallitic profiles likely represents a fixation artefact—not a reservoir of plant-available nutrients. This insight reinforces a central paradigm in tropical pedology: phosphorus availability must be interpreted through the lens of soil mineralogy, not merely extraction values [54].

From a management standpoint, this underscores the critical need for integrated diagnostic frameworks that consider pH buffering capacity, sesquioxide dominance, and P retention dynamics. Reliance on conventional extraction methods in isolation risks overlooking latent P constraints and misleading fertilization regimes. Instead, targeted interventions—such as slow-release phosphate formulations, organo-mineral amendments, or enhanced organic inputs—should be prioritized to circumvent P fixation bottlenecks and boost nutrient-use efficiency in acidic, highly weathered environments [54].

By contrast, the coastal sedimentary soils, though chemically less complex, exhibited higher CEC and BS—a reflection of their lower weathering intensity and quartz-dominated lithology [55,56]. This fertility advantage was largely confined to the upper horizon, where nutrient stocks are concentrated and vulnerable to leaching. Sodium levels remained uniformly low, consistent with expectations for tropical rainforest soils under high rainfall regimes [57], further highlighting the need for site-specific nutrient budgeting even in relatively resilient soil systems.

Together, these findings offer a scientifically grounded framework for rethinking nutrient diagnostics and fertilization strategies across lithologically diverse tropical soils, with direct implications for yield stability, ecological resilience, and sustainable land-use planning under changing climatic and socioeconomic pressures [58].

4.4 Fertility classes, degradation rates, and site-specific vulnerabilities

Both soil types evaluated in this study—coastal sedimentary sands at Kango and ferrallitic soils at Koula-Moutou—were classified as Fertility Class IV, a category denoting multiple severe agronomic constraints, particularly in pH, base saturation, and available phosphorus [37]. At Kango, very strong soil acidity and critically low phosphorus availability emerged as dominant fertility barriers. By contrast, Koula-Moutou's deeply weathered ferrallitic soils exhibited

pronounced deficiencies in exchangeable potassium (K^+) and cation exchange capacity (CEC), compounding their intrinsic nutrient limitations.

Among all variables assessed, pH stood out as a central geochemical control, exerting strong positive correlations with both CEC and Mg^{2+}/K^+ ratios ($p < 0.0001$). This relationship reinforces the role of acid–base equilibria in regulating nutrient retention and availability in tropical soils—a dynamic particularly sensitive to both parent material and land-use intensity [59–60].

The dual-index framework of Soil Degradation Rates (SDR) and Vulnerability Potential (Vp) provided deeper insight into the degradation–resilience continuum of these soils [61]. Ferralitic soils, despite registering a higher Cumulative Rating Index (CRI = 46/20), displayed greater degradation but lower vulnerability, associated with their already impoverished baseline and structural robustness. By contrast, coastal sands, though moderately degraded (CRI: 37/29), exhibited greater vulnerability to further fertility loss, underlining their tenuous nutrient balance and elevated sensitivity to anthropogenic disturbance.

These findings resemble broader pedological observations from humid tropical soils in Central Africa, where ferralitic soils are often described as chemically exhausted but physically resilient, whereas sedimentary soils are more chemically responsive yet structurally fragile under unsustainable land use [62–63]. Such distinctions carry tangible implications for regional land-use planning: ferralitic systems demand nutrient rehabilitation and pH correction, whereas coastal sands require immediate protection from accelerated degradation through conservation-based practices. In both cases, failure to act could precipitate irreversible losses in soil function and landscape productivity, compromising long-term sustainability in the Congo Basin [58,63].

4.5 Agroecological implications and sustainable soil use

The contrasting genesis and mineralogical composition of ferralitic and coastal sedimentary soils point to divergent agroecological pathways, each demanding distinct strategies for sustainable land management [7]. Ferralitic soils, enriched in sesquioxides and kaolinite clays, are chemically constrained—they exhibit low CEC, poor nutrient retention, and intense phosphorus fixation, all of which limit their agricultural potential [13, 27, 64]. By contrast, coastal sedimentary sands, though structurally fragile, remain more pedogenically dynamic and

responsive to well-targeted interventions, making them more amenable to sustainable intensification [15,65,66].

As for land-use planning, these findings carry critical implications for resource allocation, restoration priorities, and climate-smart agriculture in humid tropical systems. In ferrallitic soils, fertility restoration must begin with acid amelioration (liming) to improve nutrient availability, coupled with site-specific phosphorus supplementation and organic carbon enhancement through agroforestry, mulching, or legume-based rotations. Without such targeted efforts, these soils are unlikely to support long-term productivity under conventional cropping systems.

In coastal sand zones, the moderate inherent fertility, greater porosity, and higher management responsiveness offer a strategic entry point for sustainable agricultural intensification. Approaches that combine nutrient inputs with water conservation techniques can enhance productivity while mitigating risks of nutrient leaching and structural decline.

The divergent biogeochemical constraints and opportunities presented by these soils call for a lithologically informed, precision-based approach to soil governance—one that recognizes the legacy of Earth surface processes while designing future-facing agricultural systems adapted to local realities.

4.6 Pedoclimatic constraints and soil governance in humid tropical Central Africa

The intense equatorial rainfall [67,68], lithological heterogeneity [69], complex vegetation mosaics [70], and inherently fragile tropical soils [71] make Central Africa a key degradation hotspot within the Congo Basin's dynamic geosphere–biosphere interface. This biophysical fragility is increased by unsustainable land-use trajectories, notably slash-and-burn cultivation, abbreviated fallow periods, and unregulated deforestation—all of which disrupt soil–vegetation feedbacks, destabilize aggregate structure, and impair pedogenetic function [72].

The impacts are acute in landscapes where human disturbance has already reshaped forest architecture and floristic composition, resulting in declining aboveground biomass inputs, accelerated nutrient loss, and functional degradation of soil biota and structure [73]. These coupled anthropogenic–pedoclimatic pressures require geologically informed and spatially differentiated soil governance frameworks, capable of maintaining ecosystem services and enhancing resilience across humid tropical regions [74].

The present findings demonstrate that soil resilience is co-regulated by pedoclimatic context and land cover dynamics, highlighting the integrated influence of climate, parent material, and vegetation on soil system stability [75].

Three recommendations can be offered:

- i. Recarbonize soils using organic amendments, cover crops, and agroforestry to improve structure, biodiversity, and SOM stocks [76];
- ii. Apply lime judiciously to correct soil acidity, improve nutrient availability, and reduce Al toxicity [76-78];
- iii. Implement Conservation Agriculture: minimal tillage, crop rotation, and residue management to reduce physical and chemical degradation [78].

These actions fall within broader land governance frameworks integrating soil health and national climate strategies [7,9], such as the Congo Basin's Nationally Determined Contributions (NDCs) under the Paris Agreement. Technical solutions to local systems and socio-economic realities need participatory monitoring systems and farmer-led soil management initiatives. Geological, pedological, and ecological data enhance our understanding of soil system evolution in humid tropical Africa. Our study provides a geoscientific foundation for sustainable land-use planning in nutrient-poor yet biodiversity-rich landscapes such as the Congo Basin. The study seeks to model Earth surface processes, support agroecological resilience, and inform policy.

5. Strengths and limitations of the study

A central strength of this work is its juxtaposition of two geopedologically contrasting systems within the humid tropics of Central Africa: the highly weathered ferralitic soils of the Chaillu Massif and the quartz-rich coastal sedimentary sands of the Atlantic basin. This approach describes a natural experiment in which lithological heritage and pedoclimatic context can be distinguished in regard to soil fertility, structural stability, and degradation. By integrating physical, chemical, and functional soil metrics—including composite indices such as SDR, Vp, and CRI—the study is a multidimensional view of soil system performance and resilience. The use of high-resolution vertical profiling, with replicated samples across depth intervals, further strengthens the analysis by revealing depth-dependent patterns in nutrient distribution, acidity buffering, and structural stability that are critical for sustainable management interventions.

The process-based interpretation frames observed differences not merely as site-specific attributes but as expressions of deep-time geological inheritance interacting with contemporary climatic forcing. Accordingly, the findings are transferable to broader questions of Earth surface process modelling, agroecological planning, and land-use governance in other lithologically diverse tropical landscapes. The direct policy relevance of the results—particularly their applicability to climate-smart agriculture, national soil restoration strategies, and biodiversity conservation in the Congo Basin—underscores the value of such an approach.

Nonetheless, there are limitations. The temporal scope of the study is two discrete sampling campaigns within a single climatic year. Although mineralogical interpretations are grounded in geological mapping and prior regional studies, the absence of direct mineral phase analyses (e.g., X-ray diffraction or spectroscopic techniques) restricts the precision with which sesquioxide dominance, clay mineral assemblages, and phosphorus-binding phases can be quantified. Furthermore, the land-use context examined—predominantly secondary forest—does not encompass the full range of agricultural or degraded states in which these soils function, limiting the generalizability of the management recommendations.

Methodologically, the reliance on Olsen extraction for available phosphorus under strongly acidic conditions introduces the risk of overestimating plant-available P in ferralitic soils, given the known fixation effects of Fe and Al sesquioxides. Finally, although the two sites capture a sharp litho-climatic contrast, they do not represent the entire pedological spectrum of Gabon or the Congo Basin; extrapolating the findings to other geopedological zones requires additional site-specific validation.

By recognizing these limitations as well as the strengths, the work maintains both scientific rigour and transparency, providing a robust framework for future research aimed at refining lithology-informed soil governance in the humid tropics.

6. Conclusion

This study reveals that the fertility and degradation of tropical soils in humid Gabon are not merely products of climate or land use alone, but are fundamentally affected by the deep-time imprint of lithological inheritance and its interaction with pedoclimatic regimes. Through a comparative analysis of two contrasting geopedological systems—the quartz-rich coastal sedimentary sands at Kango and the highly weathered ferralitic soils over crystalline basement

at Koula-Moutou—we demonstrate that soil mineralogy, particle-size distribution, and acidity buffering capacity are key regulators of nutrient availability, structural stability, and resilience to degradation.

Ferralitic soils, shaped by prolonged weathering of Archean granito-gneissic substrates, exhibit severe chemical constraints, including extreme acidity, very low cation exchange capacity, and strong phosphorus fixation driven by sesquioxide dominance. These properties reinforce their low intrinsic fertility and limited responsiveness to conventional inputs, but also confer a certain structural stability that tempers their vulnerability under low-input systems.

By contrast, coastal sedimentary sands—derived from younger, less weathered sedimentary formations—are more chemically responsive, with moderate organic carbon content, higher base saturation, and greater nutrient mobility. Yet, their fragile structure and reduced water-holding capacity render them highly susceptible to nutrient leaching and degradation, particularly under intensified or poorly managed land use.

These findings validate our central hypothesis: that geologically inherited mineral and structural properties are the main drivers of soil fertility outcomes and degradation susceptibility in tropical environments. The divergent degradation indices—with higher degradation but lower vulnerability in ferralitic soils, and lower degradation but higher vulnerability in coastal sands—highlight the need for geologically contextualised soil governance.

More broadly, this work underscores the critical role of parent material in shaping Earth surface processes, including nutrient cycling, carbon sequestration, and soil structural evolution. It advances our understanding of how tropical landscapes function—and fail—under anthropogenic pressure, with direct implications for land-use policy, climate adaptation, and sustainable development.

In future, soil management strategies in Gabon and across the Congo Basin must transition from generic interventions to lithology-informed and site-specific frameworks. In ferralitic zones, restoration efforts should prioritize soil recarbonization (e.g. through agroforestry or legume rotations), pH correction via liming, and strategic phosphorus amendments adapted to high fixation environments. For coastal sandy systems, the focus should shift toward integrated nutrient and water management, alongside erosion control and organic matter stabilization.

These recommendations, if they are accepted for national land-use planning and climate adaptation strategies, can ensure that soil stewardship aligns with agroecological resilience, biodiversity conservation, and regional sustainability goals. In the context of accelerating climate change, it is clear that strengthening soil system resilience will require strategies that address both the inherited geological constraints and the evolving pedoclimatic pressures shaping tropical landscapes. Soil management strategies in Gabon and across the Congo Basin must transition from generic to integrated, lithology-informed frameworks that combine adaptive nutrient management, organic matter restoration, and structural conservation measures for each soil system's vulnerabilities and strengths.

Credit Author Statement

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Supplementary material

Supplementary Table 1. Mean soil physical properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	n	Depth (cm)	Clay (%)	Sand (%)	Silt (%)	Silt/Clay	Texture	Bd (g/cm)	ISS (%)	WC (%)	AP (%)	TP (%)
Coastal sedimentary sands	21	0-10	8.09 ± 6.27 ^a	59.04 ± 11.70 ^a	32.86 ± 9.32 ^a	5.78 ± 3.35 ^a	Sandy loam	1.43 ± 0.01 ^a	14.62 ± 7.82 ^a	25.98 ± 4.31 ^a	19.97 ± 4.50 ^a	45.95 ± 0.48 ^a
		10-20	13.90 ± 7.96 ^{ab}	49.33 ± 8.70 ^{bc}	36.76 ± 9.53 ^a	4.23 ± 3.73 ^a	Loam	1.44 ± 0.01 ^{ab}	7.27 ± 3.32 ^{bc}	22.60 ± 3.65 ^{ab}	22.96 ± 3.61 ^a	45.55 ± 0.56 ^{ab}
		20-30	20.57 ± 7.82 ^{bc}	48.10 ± 10.51 ^b	31.33 ± 9.84 ^a	1.84 ± 1.15 ^b	Loam	1.45 ± 0.02 ^{ab}	5.28 ± 1.95 ^b	22.57 ± 3.57 ^{ab}	22.75 ± 3.18 ^a	45.32 ± 0.86 ^{ab}
Ferralitic soils	18	0-10	19.67 ± 9.39 ^{bc}	66.89 ± 10.87 ^a	13.44 ± 5.81 ^b	0.98 ± 0.83 ^b	Sandy loam	1.46 ± 0.04 ^b	24.15 ± 16.07 ^d	24.93 ± 6.72 ^a	19.94 ± 6.78 ^a	44.86 ± 1.39 ^b
		10-20	27.11 ± 9.51 ^{cd}	61.44 ± 8.97 ^a	11.44 ± 5.22 ^b	0.68 ± 0.87 ^b	Sandy clay loam	1.49 ± 0.03 ^c	13.00 ± 7.39 ^{ac}	21.90 ± 4.22 ^{ab}	21.71 ± 4.22 ^a	43.61 ± 1.07 ^c
		20-30	31.89 ± 8.64 ^d	57.56 ± 8.5 ^{ac}	10.56 ± 4.38 ^b	0.40 ± 0.29 ^b	Sandy clay loam	1.49 ± 0.02 ^c	6.56 ± 3.96 ^{bc}	19.79 ± 3.56 ^{ab}	23.91 ± 3.57 ^a	43.71 ± 0.76 ^c

n : Number of samples ; Bd: Bulk density ; ISS : Soil aggregate stability index ; WC : Water content ; AP : Air porosity ; TP : Total porosity. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.

Supplementary Table 2. Mean soil chemical properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	n	Depth (cm)	pH	SOM (%)	SOC (%)	TN (%)	C/N	NO_3^- (ppm)	NH_4^+ (ppm)
Coastal sedimentary sands	21	0-10	4.75 ± 0.33 ^a	5.38 ± 1.71 ^a	3.12 ± 1.00 ^a	0.29 ± 0.10 ^{ab}	11.24 ± 2.66 ^a	16.60 ± 7.70 ^a	37.84 ± 18.65 ^a
		10-20	4.56 ± 0.21 ^a	3.51 ± 1.06 ^{bc}	2.03 ± 0.62 ^{bc}	0.23 ± 0.06 ^{bc}	8.95 ± 1.80 ^b	16.06 ± 6.19 ^a	30.21 ± 9.02 ^{ab}
		20-30	4.52 ± 0.20 ^a	2.63 ± 0.76 ^c	1.53 ± 0.44 ^c	0.21 ± 0.07 ^{cd}	7.82 ± 1.94 ^b	15.72 ± 6.38 ^a	29.87 ± 9.95 ^{ab}
Ferralitic soils	18	0-10	3.63 ± 0.52 ^b	6.65 ± 1.51 ^d	3.86 ± 0.87 ^d	0.35 ± 0.13 ^a	11.77 ± 2.23 ^a	17.94 ± 7.35 ^a	40.33 ± 23.64 ^a
		10-20	3.78 ± 0.46 ^b	4.70 ± 1.76 ^{ab}	2.72 ± 1.03 ^{ab}	0.22 ± 0.1 ^{bd}	12.89 ± 3.15 ^a	13.97 ± 5.24 ^a	28.55 ± 10.33 ^{ab}
		20-30	3.86 ± 0.33 ^b	2.60 ± 1.02 ^c	1.51 ± 0.60 ^c	0.14 ± 0.06 ^d	11.57 ± 2.90 ^a	11.90 ± 5.56 ^a	22.34 ± 6.00 ^b

n : Number of samples ; SOM: Soil organic matter ; TN : Total nitrogen ; C/N : Carbon-to-nitrogen ratio ; NO_3^- : Nitrate ; NH_4^+ : Ammonium. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.

Supplementary Table 3. Mean soil fertility properties of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	n	Depth (cm)	av.P (ppm)	Ca ²⁺ (cmol/kg)	Mg ²⁺ (cmol/kg)	K ⁺ (cmol/kg)	Na ⁺ (cmol/kg)	CEC (cmol/kg)	SBE (cmol/kg)
Coastal sedimentary sands	21	0-10	6.27 ± 3.08 ^{ab}	5.62 ± 3.48 ^a	1.82 ± 0.88 ^a	0.37 ± 0.15 ^a	0.09 ± 0.06 ^a	18.12 ± 6.28 ^a	7.91 ± 4.17 ^a
		10-20	4.66 ± 1.80 ^{ac}	3.23 ± 1.17 ^b	1.24 ± 0.53 ^b	0.29 ± 0.08 ^b	0.06 ± 0.04 ^{ab}	15.77 ± 4.20 ^{ab}	4.82 ± 1.55 ^b
		20-30	3.31 ± 1.38 ^c	2.55 ± 1.02 ^b	0.95 ± 0.55 ^b	0.29 ± 0.09 ^b	0.05 ± 0.04 ^{ab}	16.31 ± 4.58 ^{ab}	5.17 ± 6.64 ^{ab}
Ferralitic soils	18	0-10	11.87 ± 4.66 ^d	0.92 ± 0.53 ^c	0.40 ± 0.32 ^c	0.18 ± 0.07 ^c	0.05 ± 0.05 ^{ab}	12.57 ± 5.38 ^{bc}	1.54 ± 0.84 ^c
		10-20	7.60 ± 2.89 ^b	0.60 ± 0.36 ^c	0.21 ± 0.14 ^c	0.10 ± 0.05 ^{cd}	0.04 ± 0.04 ^b	8.35 ± 3.00 ^{cd}	0.95 ± 0.50 ^c
		20-30	5.11 ± 0.93 ^{bc}	0.43 ± 0.52 ^c	0.12 ± 0.08 ^c	0.06 ± 0.03 ^d	0.03 ± 0.04 ^b	5.34 ± 2.00 ^d	0.65 ± 0.61 ^c

n : Number of samples ; av.P : Available phosphorus; Ca²⁺ : Exchangeable calcium; Mg²⁺ : Exchangeable magnesium; K⁺ : Exchangeable potassium; Na⁺ : Exchangeable sodium; CEC: Cationic exchangeable capacity; SBE : Sum exchangeable bases. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.

Supplementary Table 4. Mean soil fertility proxies of studied soil types: Coastal sedimentary sands (Kango site) and Ferralitic soils (Koula-Moutou site)

Soil types	n	Depth (cm)	BS (%)	Ca ²⁺ :Mg ²⁺	Mg ²⁺ :K ⁺
Coastal sedimentary sands	21	0-10	43.66 ± 14.32 ^a	3.16 ± 1.13 ^a	4.87 ± 1.62 ^a
		10-20	31.55 ± 10.03 ^{ab}	2.98 ± 1.77 ^a	4.32 ± 1.49 ^a
		20-30	32.00 ± 38.95 ^{ac}	3.01 ± 1.17 ^a	2.97 ± 1.34 ^b
Ferralitic soils	18	0-10	15.91 ± 14.00 ^{bc}	2.65 ± 1.11 ^a	2.22 ± 1.40 ^b
		10-20	13.17 ± 9.07 ^b	3.20 ± 1.29 ^a	2.04 ± 0.95 ^b
		20-30	14.02 ± 12.88 ^{bc}	3.10 ± 1.31 ^a	2.00 ± 1.17 ^b

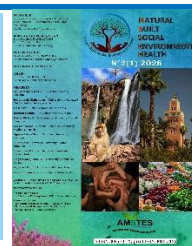
n: Number of samples ; BS : Base saturation; Ca²⁺/Mg²⁺ : Calcium-to-Magnesium ratio; Mg²⁺/K⁺ : Magnesium –to-Potassium ratio. Subscript letters following the data denote statistical similarities and differences among groups. Identical letters indicate that the compared groups are not significantly different. Distinct subscripts represent statistically significant differences in mean values, as determined by Tukey's multiple comparison test at the 5% significance level.



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Integrated Algal–Microbial Consortia for Wastewater Treatment: Mechanisms, Performance and Future Perspectives

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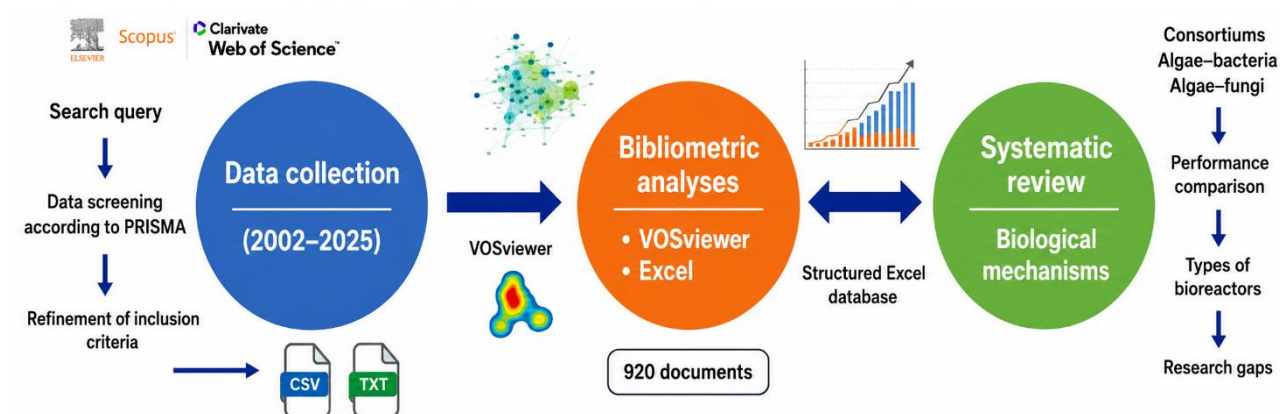
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Graphical Abstract

This study adopts a systematic PRISMA-based methodology to collect and screen scientific articles from 2002 to 2025 using the main databases Scopus and Web of Science. Bibliometric studies were performed using VOSviewer and Excel to analyze research trends, keyword mapping, and publication patterns from a chosen set of 920 documents. Next, the review integrates the latest understanding of algal-microbial consortia, contrasts their biological mechanisms, efficiencies, identifies the types of reactors used, and pinpointing the gaps in the research field.



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Abstract: Algae, especially microalgae and macroalgae, are photosynthetic organisms that have been used for environmental purposes for a long time, which has been covered in depth through research. The major environmental uses of wastewater treatment, including nutrient recovery and reduction of the organic load, are mentioned in the literature. Microalgae are characterized by a very rapid growth, a huge potential to assimilate nitrogen and phosphorus, and an ability to form symbiotic relationships with other microorganisms, whereas macroalgae are mainly recognized for their high biomass production and their capacity to absorb pollutants in the water environment. This review paper has adopted the PRISMA method to strictly select research papers on biological consortia of the following: microalgae, bacteria, microalgae–fungi, macroalgae, bacteria, and macroalgae–fungi. Assessment of the chosen articles indicates that the cohabitation of microalgae and bacteria is the most favored theme in the literature by far, given its well-documented effectiveness and biological stability. In addition, bibliometric analyses show that there has been increasing attention to microalgae–fungi consortia since 2015, indicating new trends towards biofloc formation, which greatly facilitates biomass separation. On the other hand, consortia involving macroalgae in association with microorganisms (either bacteria or fungi) are still in their infancy in research.

Keywords: *Agricultural mulching films, Recycling barriers, Circular economy.*

Introduction

Increasing industrialisation, combined with rapid population growth, has led to large volumes of untreated or inadequately treated wastewater being discharged into the natural environment, particularly in developing regions [1]. Industrial discharges containing nutrients, heavy metals, and toxic organic pollutants contribute significantly to eutrophication, ecosystem degradation, and declining water quality [2]. Beyond environmental concerns, water pollution has a profound impact on human health, leading to waterborne diseases, gastrointestinal infections, and chronic conditions resulting from prolonged exposure to pollutants such as lead, cadmium, and persistent organic pollutants [3]. It is therefore essential that we develop effective, sustainable, and environmentally friendly wastewater treatment solutions, not only to preserve our planet but also to protect our health. It is in this context that algae emerge as a natural and promising solution. Indeed, algae are rich in bioactive compounds such as pigments, proteins, polysaccharides, and lipids. These compounds are particularly valuable as they enhance the economic potential of algae and expand the prospects for biomass utilisation. In particular, their application in wastewater treatment has attracted increasing attention over the last few decades. Both microalgae and macroalgae exhibit a remarkable ability to remove inorganic nutrients such as nitrogen and phosphorus, reduce the organic load, and absorb certain heavy metals and micropollutants present in effluents [4,5]. Moreover, through photosynthesis, algae release oxygen, which in turn enhances biological oxidation processes and thus reduces the energy requirements for mechanical aeration in conventional wastewater treatment systems [6].

Numerous species of microalgae such as *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Tetraselmis suecica* have shown great potential in the treatment of different types of wastewater, such as urban and industrial effluents [7,8]. In parallel, a number of marine macroalgae including *Ulva lactuca*, *Gracilaria* spp., and *Laminaria* spp. have demonstrated a high purification capacity, especially for high salinity effluents and coastal regions [9,10].

Nevertheless, the use of algae in monoculture also has some disadvantages, such as their sensitivity to environmental changes; in some instances, they are not sufficiently efficient for the complete removal of organic matter and total nitrogen [11]. To increase the efficiency of algal systems, new approaches have been developed based on the mixed culture of algae with other microorganisms such as bacteria and fungi. These biological consortia rely on symbiotic interactions and metabolic complementarity, in which photosynthetic organisms produce oxygen and assimilate nutrients, whereas bacteria and fungi degrade complex organic matter, generate carbon dioxide, and release compounds that can be subsequently used by the photosynthetic organisms [12].

Algae–bacteria systems have been widely studied and have progressively demonstrated high effectiveness in achieving the simultaneous removal of organic matter, nitrogen, and phosphorus, as well as reducing the energy demand of wastewater treatment [13]. Recently, algae–fungi consortia have also come into focus, with a growing interest in the form of bioflocs, which facilitate biomass harvesting and increase the stability of treatment processes [14]. These combined strategies represent a promising path towards the development of wastewater treatment technologies that are highly efficient, sustainable, and economically feasible.

Despite the growing body of literature on algal-based wastewater treatment, existing reviews have predominantly focused on microalgae–bacteria systems, with limited attention given to macroalgae-based consortia and tripartite algae–bacteria–fungi interactions. To date, no comprehensive review has comparatively synthesised microalgae–bacteria, microalgae–fungus, macroalgae–bacteria, and macroalgae–fungus systems within a unified analytical framework spanning the past two decades. Addressing this gap, the present review provides a structured comparative analysis of the four principal algal–microorganism consortia, evaluates their pollutant removal performance and operational mechanisms, identifies key technological and biosafety challenges, and proposes strategic directions for large-scale and sustainable implementation. Based on a combined systematic review and bibliometric analysis using the Scopus and Web of Science databases, this study identifies major scientific trends, dominant research themes, knowledge gaps, and future research priorities in this rapidly expanding field.

Bibliometric analysis of algae-microorganism consortia for wastewater treatment

Data Sources

The bibliographic data used in this study were extracted from two databases, Scopus and Web of Science, to obtain accurate and representative information on research related to wastewater treatment using biological consortia involving microalgae, macroalgae, bacteria, and fungi. Publications were identified using a query combining keywords related to algae, associated microorganisms, and the field of wastewater treatment within the Title–Abstract–Keywords fields: (microalga OR macroalga OR algae* OR seaweed*) AND (bacteria* OR bacterial* OR fungi* OR fungal*) AND (wastewater* OR wastewater treatment* OR effluent*). The search was conducted between 2002 and 2025 to document the evolution of the field’s research. Both articles and review papers were selected to provide a thorough understanding of research trends. After that, the bibliographic data were exported and analysed using VOSviewer.

Selection Criteria

We performed the systematic literature review according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [25]. We conducted the first comprehensive bibliographic review of the literature on the biological treatment of wastewater by microalgae and macroalgae, in consortia with bacteria and fungi, comparing their mechanisms of action, benefits, and drawbacks. Precise inclusion and exclusion criteria were necessary in order to define the research question, reduce selection bias, and ensure scientific homogeneity of the studies. We used peer-reviewed journal articles published between 2002 and 2025. This allowed the examination of both early and recent stages in the development of this research area.

The use of 2002 as the starting date was deliberately selected because the early 2000s marked a period when more organised studies on algal–microbial consortia began to emerge, alongside advances in molecular biology, environmental biotechnology, and wastewater treatment engineering. Before this period, studies were relatively scarce and predominantly focused on monocultures rather than on integrated systems involving macroalgae or microalgae in association with bacteria and fungi. Excluding publications prior to 2002 therefore ensures methodological consistency and focuses the analysis on the phase during which consortium-based approaches became scientifically and technologically relevant. The review was extended to 2025 to include the most recent developments and emerging trends in macroalgae–bacteria–fungi and microalgae–bacteria–fungi systems. The search was mainly restricted to English and French publications, and articles in other languages, conference abstracts without full text, editorial pieces, and non-scientific documents were excluded. In addition, studies that did not explicitly address wastewater treatment or that did not involve algal–microorganism consortia were discarded. The use of these criteria resulted in a final collection of relevant publications for qualitative and bibliometric analyses.

Inclusion and Exclusion Criteria for Articles

The aim of this research was to investigate patterns of wastewater treatment research using biological consortia of microalgae and macroalgae with bacteria and fungi, through the analysis of studies conducted during the period from 2002 to 2025. The bibliographic search was carried out in the Scopus, ScienceDirect, and Web of Science databases, which are major and reliable sources in the fields of environmental science and biotechnology. Firstly, the total number of articles recorded was 3,231, which were then downloaded in CSV and plain-text file formats for easier

handling and bibliometric analysis. Subsequently, the titles and abstracts of all publications were reviewed to further evaluate their suitability against the inclusion and exclusion criteria that had been set in advance (Table 2). To be included, articles had to explicitly address, either wholly or partially, the use of algae–microorganism consortia in the context of wastewater treatment, bioremediation, or nutrient and pollutant removal.

The screening step led to the rejection of 2,311 papers that did not meet these requirements. In addition, a number of papers examined wastewater treatment using algae or micro-organisms as single components, thus ignoring the interactions within biological consortia, which constituted the basis for their exclusion. After this selection, a final set of 920 articles was chosen for a detailed qualitative and bibliometric study.

Table 1. Inclusion and Exclusion Criteria for the Systematic Review

	Inclusion Criteria	Exclusion Criteria
Publication period	Articles published between 2002 and 2025.	Articles published before 2002, except for a few important theoretical references.
Thematic relevance	Studies explicitly addressing wastewater treatment using biological consortia involving algae–microorganisms, as mentioned in the title, abstract, or keywords.	Articles not directly related to the keywords of interest.
Languages	Publications written in English or French.	Publications in languages other than English or French.
Document types	Peer-reviewed scientific articles, including research articles and literature reviews.	Conference abstracts without full text, reports, or non-peer-reviewed documents.
Duplicates	Only one version retained when the same article is indexed in multiple databases.	Duplicate articles identified across different databases.

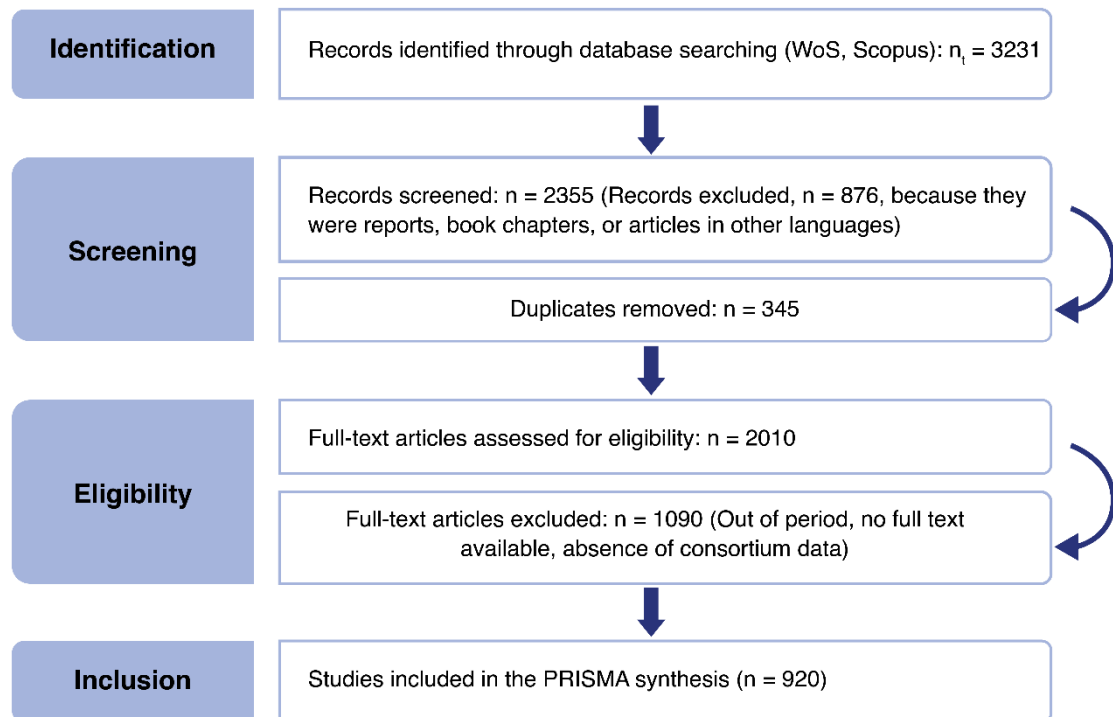
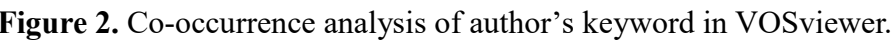


Figure 1. PRISMA 2020: flow diagram for new systematic review which included searches of databases and registers

Interactions between microalgae, macroalgae, fungi, and bacteria

The keyword co-occurrence analysis conducted using VOSviewer highlights the thematic structuring of research on wastewater treatment through biological consortia involving algae and micro-organisms. The network is dominated by the terms “wastewater treatment” and “microalgae”, which occupy a highly connected central position linked to “bacteria”, “biomass”, *Chlorella vulgaris*, “nitrogen”, “nitrate”, “carbon fixation”, and “microalgae–bacteria consortia”. This shows that microalgae–bacteria consortia represent the main axis and the most mature area of the literature, especially in terms of nutrient uptake and improved purification performance.

In contrast, words related to fungi (e.g. fungi, *Aspergillus*, co-cultivation, symbiosis) occur more peripherally and less often, indicating that there is still a significant degree of unexplored potential for the use of algae–fungi consortia as flocculating entities with enzymatic activity and biodegradation capacity. Furthermore, macroalgae (e.g. macroalgae, *Ulva*, seaweed) occupy a marginal position within the network, showing weak connections to the central core. This suggests a major scientific gap regarding macroalgae–bacteria and macroalgae–fungi consortia.



Most productive and collaborative countries

Research on wastewater treatment using algal consortia (microalgae and macroalgae associated with bacteria or fungi) is embedded within a structured international collaborative network, as revealed by a bibliometric analysis using VOSviewer software (Figure 3). The most productive countries are grouped into several interconnected clusters, reflecting the existence of active transnational scientific collaborations. The proximity of countries within the network indicates the intensity of their collaborations, with the strongest links observed between nations demonstrating

high scientific output. China emerges as the leading scientific driver, with a high and recent publication output, closely connected to India, Japan, and the United States. The latter plays a central role as a collaborative platform linking Asia, Europe, and Latin America. In Europe, Spain is prominent in terms of the extent of collaborations and contributions made to algal bioremediation research.

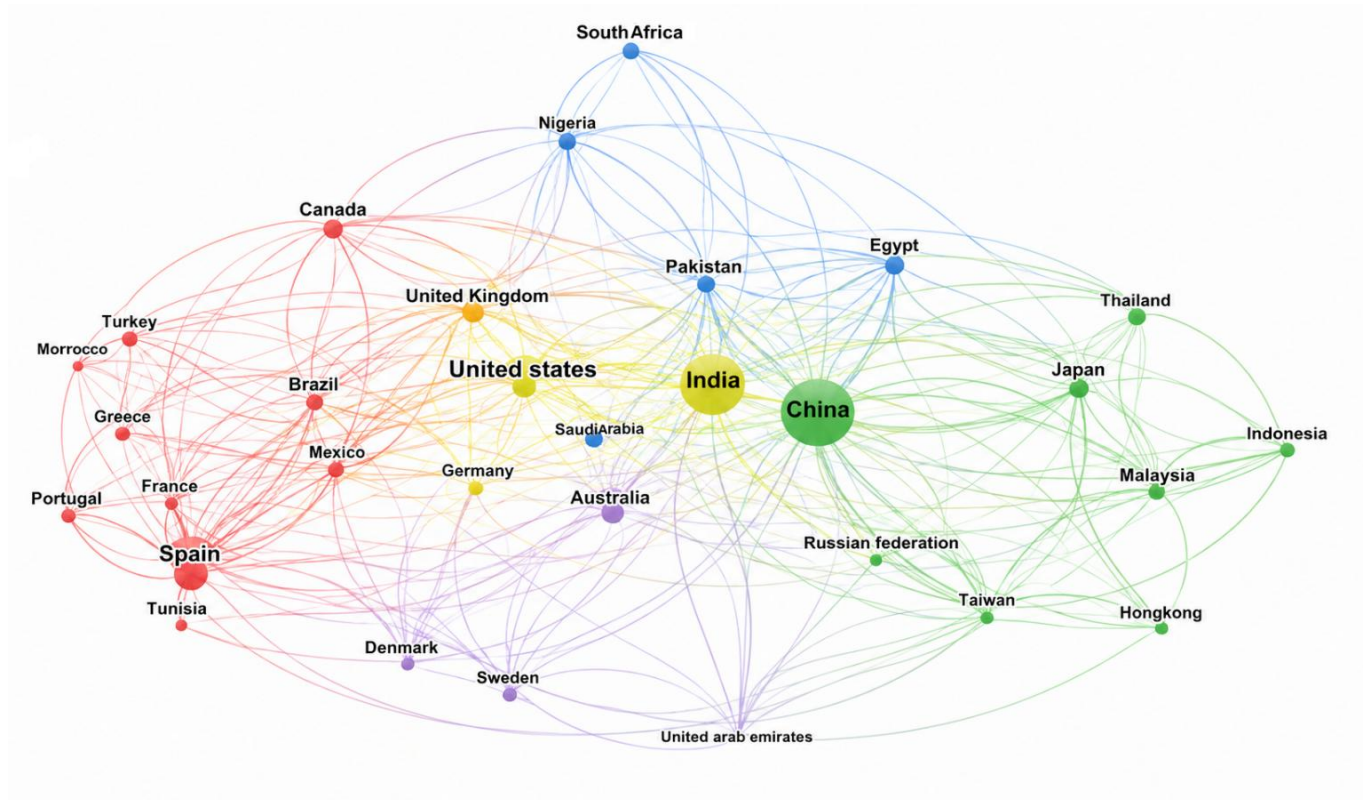


Figure 3. Publication distribution of reviewed articles by country

In contrast, African countries and some parts of the Global South are severely underrepresented, reflecting a geographical imbalance and an under exploitation of scientific potential. This discrepancy highlights the importance of strengthening international collaborations and supporting applied studies in local contexts under high environmental pressure.

Annual distribution of documents

Figure 4 depicts a steady and significant growth in publications over the 2002–2025 period covering algae–microorganism consortia. The quantity of studies was very low during the 2002–2012 timeframe, and scientists were considered to be in an exploratory phase of research. From 2013 onwards, and more significantly after 2015, the number of publications increased rapidly; microalgae–bacteria consortia have dominated the field, accounting for over 1,100 publications,

and represent the primary research focus, especially in wastewater treatment and biomass valorisation.

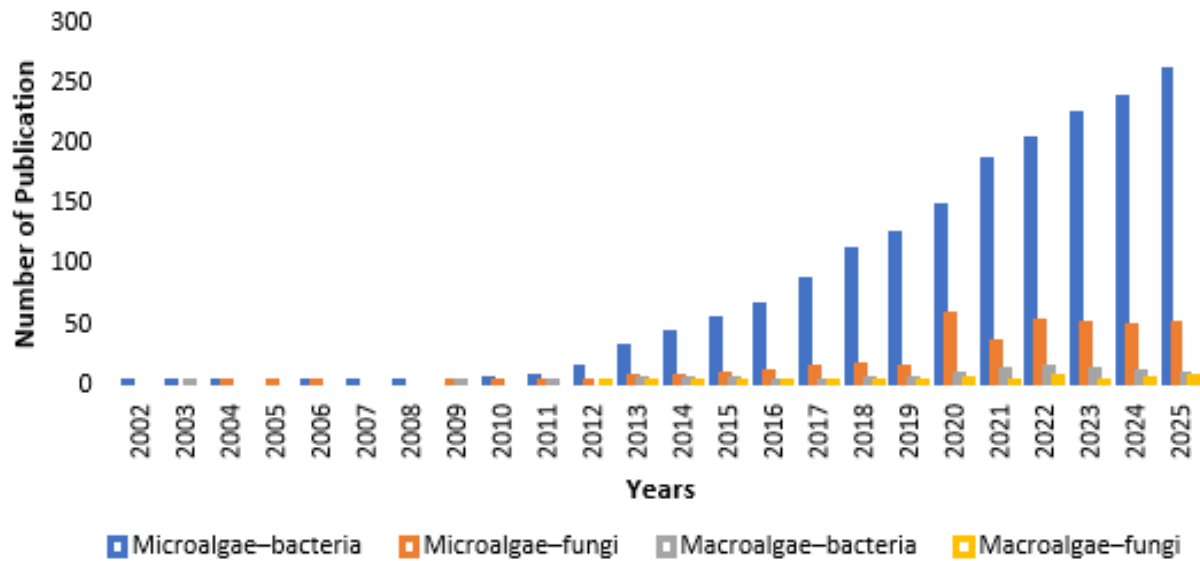


Figure 4. Evolution of the Numbers of publications on algae × microorganisms consortia (2002–2025).

On the other hand, macroalgae consortia remain a niche research area that is still underexplored, and are therefore becoming an emerging field. Similarly, for microalgae–fungi consortia, a recent rise in the number of papers has been observed, reflecting an increasing interest in microbial combinations and the production of metabolites for biotechnological applications.

General Overview

Definition

The term algae has been used since ancient times and was first introduced by Carl Linnaeus in 1753. Subsequently, in 1789, Antoine Laurent de Jussieu established a classification of plants and defined algae in relation to the entire plant kingdom, thereby establishing their current taxonomic status in botany [15]. The scientific discipline dedicated to the study of algae is known as phycology [16].

Algae are a group of photosynthetic organisms, meaning they convert light energy into chemical energy through the process of photosynthesis. They are found in aquatic environments and may be microscopic, referred to as “microalgae”, or macroscopic, known as “macroalgae”. The latter are attached to substrates in coastal areas, are visible to the naked eye, and can reach several meters in length. The term “seaweed” is generally used to refer to benthic marine algae (macroalgae). These

macroalgae are defined as multicellular eukaryotic organisms exhibiting considerable morphological diversity.

To identify a marine macroalga, phycologists primarily examine its appearance and its environment. For identification at a more specific level, the most relevant attributes are mainly colour, shape, thallus configuration, and the possible presence of calcification. In certain cases, information relating to reproduction and the structure of reproductive organs is also required. In order to correctly identify biological material, detailed microscopic examination of vegetative and reproductive characteristics is necessary [17].

Microalgae, on the other hand, live suspended in water (from the surface down to the seabed). Often called phytoplankton, microalgae are unicellular or undifferentiated multicellular organisms. Under this designation, they belong either to the Eukaryotes or the Prokaryotes, the latter being represented mainly by cyanobacteria, also known as blue-green algae. Their size ranges from a few micrometres to around one hundred micrometres. Observation therefore requires the use of an optical or electron microscope [18]. Despite this morphological diversity, they share common biological characteristics. To date, more than 156,000 species and subspecies of algae have been described worldwide [19].

General Characteristics

Cytological and Biochemical Features

Algal cells are surrounded by a rigid cell wall [16,20]. In many cases, this wall is composed of a network of cellulose fibres. Other polysaccharides such as pectin, xylans, mannans, alginates, and fucoidans may also be present [21]. All algae contain chlorophyll a. Some species also contain other types of chlorophyll, which differ slightly from chlorophyll a [21]. These chlorophylls are associated with numerous additional accessory pigments, such as carotenoids and, more rarely, phycobiliproteins, which give the different algal groups their distinctive colours [22].

Mode of nutrition

Algae, like the majority of plants, absorb light energy through pigments (chlorophyll and others) located in plastids in eukaryotes or dispersed in the hyaloplasm in prokaryotes (cyanobacteria). Through chlorophyll a, this energy is converted into chemical energy; this process is known as photosynthesis. It is used in the production of carbon-based organic compounds from mineral

elements (CO_2 , H_2O) extracted from the environment, which makes algae autotrophic organisms. This series of reactions leads to the release of oxygen.

The gaseous exchanges carried out by chlorophyll (absorption of CO_2 and release of O_2) occur in parallel with respiratory gaseous exchanges (absorption of O_2 and release of CO_2). The release of CO_2 during the night is linked to respiration; during the day, this respiratory activity is masked by photosynthetic activity, which is quantitatively greater and characterized by the release of O_2 [23,24].

Characteristics of wastewater

Water is one of the most important resources for the life of human beings, animals, and plants on planet Earth. It covers about 71% of the Earth's surface [26]. Over the last couple of decades, the shortage of freshwater has become a major issue worldwide as natural water resources are being overexploited. The Technical Report on Water and Climate Change by the United Nations and United Nations Environment Programme reveals that the world population will reach approximately 8.5 billion in 2030, 9.7 billion in 2050, and nearly 11 billion in 2100 [27].

Besides population increase, alterations in socio-economic settings and lifestyle changes leading to different consumption patterns will result in major changes in the quantity and quality of wastewater, and therefore will place new burdens on existing wastewater treatment systems, which may not be able to handle these new challenges. It is a fact that only 20% of the wastewater produced worldwide is treated adequately before it is discharged, and of that treated wastewater (TW), only 2–15% is reused for irrigation depending on the region [28]. Around 80% of the total wastewater generated worldwide is discharged without proper treatment, according to the 2017 United Nations World Water Development Report 2017 [29].

Table 2. Algal growth factors

	Category	Factor	Effect on Algal Growth	Examples / Conditions
Macroalgae	Climatic factors	Light intensity	Determines spatial distribution, biomass and thallus morphology	Photophilous algae in exposed areas; sciaphilous species in shaded areas; polymorphism in charophytes
		Temperature / seasonality	Influences seasonal dynamics, species succession and phenology	Summer thermophilic species; winter species (e.g. <i>Hydrurus foetidus</i>) disappearing above 13 °C
	Habitates Conditions	Substrate	Determines anchorage, stability and growth mode	Rhizoids (charophytes), basal discs (<i>Rhizoclonium</i>), colonisation of sediments (<i>Phormidium</i> , <i>Lyngbya</i>)
		Current velocity	Influences thallus morphology, compactness and attachment	Rheophilic species (<i>Lemanea</i>); species carried by the current (<i>Spirogyra</i>)
		Water depth	Controls light penetration and biomass stability	Optimal biomasses below 1 m; some charophytes up to 10 m
		Light	Energy source for photosynthesis; influences productivity	Light intensity, spectral quality and photoperiod are determining factors; risk of photoinhibition
Microalgae	Abiotic factors	Temperature	Controls metabolic activity and growth	Psychrophilic (<10 °C), mesophilic (10–20 °C) and thermophilic (>30 °C) species
		pH	Influences carbon assimilation and cellular stability	Optimum generally between 8.2 and 8.7; controlled by aeration or CO ₂ injection
		Salinity	Affects osmotic and ionic balance	Osmotic stress in case of sudden variation; adjustment by dilution or salt addition
		Carbon	Key element for photosynthesis	Supplied in the form of CO ₂ or bicarbonates
	Nutritional factors	Nitrogen	Essential for protein and nucleic acid synthesis	NO ₃ ⁻ , NH ₄ ⁺ , urea
		Phosphorus	Essential for nucleic acids and ATP	Soluble phosphates required
		Trace elements & vitamins	Support enzymatic functions	Mg, Ca, Mn, Zn, Cu, Mo; vitamins sometimes beneficial
		Associated micro-organisms	Competition or synergy	Presence of bacteria, fungi, viruses
	Biotic & operational factors	Hydrodynamic conditions	Affect light exposure and gas transfer	Residence time, mixing, shear stress
		Gas transfer	Controls availability of CO ₂ and O ₂	Essential in dense cultures

Table 3. Comparison of different characteristics of wastewater from different sources

Wastewater source	pH	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	Nitrogène (mg/L)	Phosphorus (mg/L)	Reference
Municipal wastewater	7.4	—	816 ± 129 (COD)	—	110 ± 16 (TN)	15.3 ± 1.3 (PO ₄ -P)	[32]
	7.2 ± 0.5	—	245.6 ± 16 (TOC)	—	101.3 ± 2.8 (TN)	5.2 ± 1.3 (PO ₄ -P)	[33]
Domestic wastewater	7.6 ± 0.3	—	411 ± 156 (COD)	—	37.0 ± 9.3 (TKN)	9.2 ± 2.6 (TP)	[34]
	7.4 ± 0.15	—	430 ± 198 (COD)	—	60 ± 11 (TKN)	8.7 ± 1.6 (TP)	[35]
Cheese industry	3.82–5.98	15,500–18,000	44,774–66,739	320.5–436.5	-	PO ₄ -P: 291–350	[36]
Dairy	4.53 ± 0.67	170 ± 121.24	1007.3 ± 224.19	299.67 ± 89.97	NH ₄ ⁺ : 5.23 ± 5.26; NO ₂ ⁻ : 0.35 ± 0.13; NO ₃ ⁻ : 25.67 ± 13.68; TKN: 16.31 ± 0.58	PO ₄ ³⁻ : 46.97 ± 33.59	[37]
	6.58 ± 0.1	11,000 ± 50	13,054 ± 5	9622 ± 2.51	NO ₃ ⁻ -N: 6.62 ± 0.43; TKN: 69.32 ± 1.01	PO ₄ ³⁻ -P: 13.12 ± 0.7	[38]
Sugar refinery	4.77–4.94	1164–14,491	11,333–357,725	300–2533	NH ₄ ⁺ : 104.98–177.3; NO ₂ ⁻ : 0.45–2.79; NO ₃ ⁻ : 127–1477; TKN: 0.66–27.07	PO ₄ ³⁻ : 46.97–1426	[36]
Sugar	7.51	1070	1526.9	1413	TKN : 4.93	4.24	[39]
Cassava starch processing	4.5–4.92	6300	10,496	827	TN: 542.5	TP: 94	[40]
Cassava biogas effluent	7.5 ± 1.0	205 ± 12.3	47.67 ± 2.36	—	—	PO ₄ ³⁻ : 23.53 ± 1.70	[41]
Fish processing	—	1128 ± 16.0	—	360 ± 15.46	NH ₃ -N: 2.0; NH ₄ ⁺ -N: ≈320; NO ₂ ⁻ -N: ≈0.56; NO ₃ ⁻ -N: ≈22.6	—	[42]
Tuna wash processing	7.38	139.15 g/L	—	23.48 ± 0.7	TKN: 18.2 ± 0.2	PO ₄ ³⁻ : 1.62 ± 0.1	[43]
Dyeing industry	—	52.4	111	—	—	—	[44]
Pharmaceutical industry	—	120	490	370	—	—	[45]
	5–9.5	—	2580–6840	89	NH ₄ -N: 64.2 NO ₃ -N : 0.24 TN :228	—	[46,47]
Fish processing industries Maroc	—	4877	10557	3537	TN : 631	84	[48]

New chemical formulations, regularly introduced into communities for use in industries, agriculture, and homes, have caused wastewater quality to deteriorate, leading to the generation of water pollutants that are hard to eliminate and often contain nutrients and pesticides. Approximately 0.1% of wastewater comprises suspended and dissolved solids, mainly made up of inorganic and non-biodegradable substances [30]. The organic matter in wastewater mainly comprises proteins, carbohydrates, and lipids derived from the wastes of agricultural and food processing industries [31].

Risks of wastewater to the environment and human health

One of the major sources of environmental pollution and health hazards is untreated wastewater, which is usually rich in organic materials, nitrogen, phosphorus, and other chemical and biological pollutants [49]. When these wastewater effluents are released directly into water bodies, they may trigger eutrophication, a phenomenon that causes rapid growth of algae and a decrease in oxygen levels, ultimately leading to serious alterations in aquatic ecosystems [50].

In addition, wastewater has the potential to harbour a variety of pathogenic microorganisms including bacteria, viruses, and parasites, which cause waterborne diseases such as diarrhoea, cholera, and hepatitis [29]. Hence, contact with such contaminated waters is a major health threat, especially in areas with limited wastewater treatment facilities, where it contributes to the perpetuation of a cycle of parasitic and chemical transmission between the environment and humans [51].

Consortia of microalgae and macroalgae with bacteria and fungi for wastewater treatment

The high efficiency of microalgae–fungi and microalgae–bacteria co-cultivation systems in wastewater treatment is primarily based on synergistic metabolic interactions among the different microorganisms. In these consortia, microalgae assimilate inorganic carbon and nutrients through photosynthesis, producing oxygen that supports the aerobic metabolism of heterotrophic microorganisms, which are responsible for the oxidation of organic matter and the release of carbon dioxide and mineralised nutrients, which promote algal growth [26].

Fungal extracellular enzymes in microalgae–fungi systems are the principal agents that break down complex organic materials and suspended solids into low-molecular-weight substrates, thus increasing nutrient bioavailability, biomass production, and biofloc formation, which facilitates harvesting [52]. The efficiency of these systems relies heavily on the initial inoculation ratio, which determines the growth balance and the degree of pollutant removal [52].

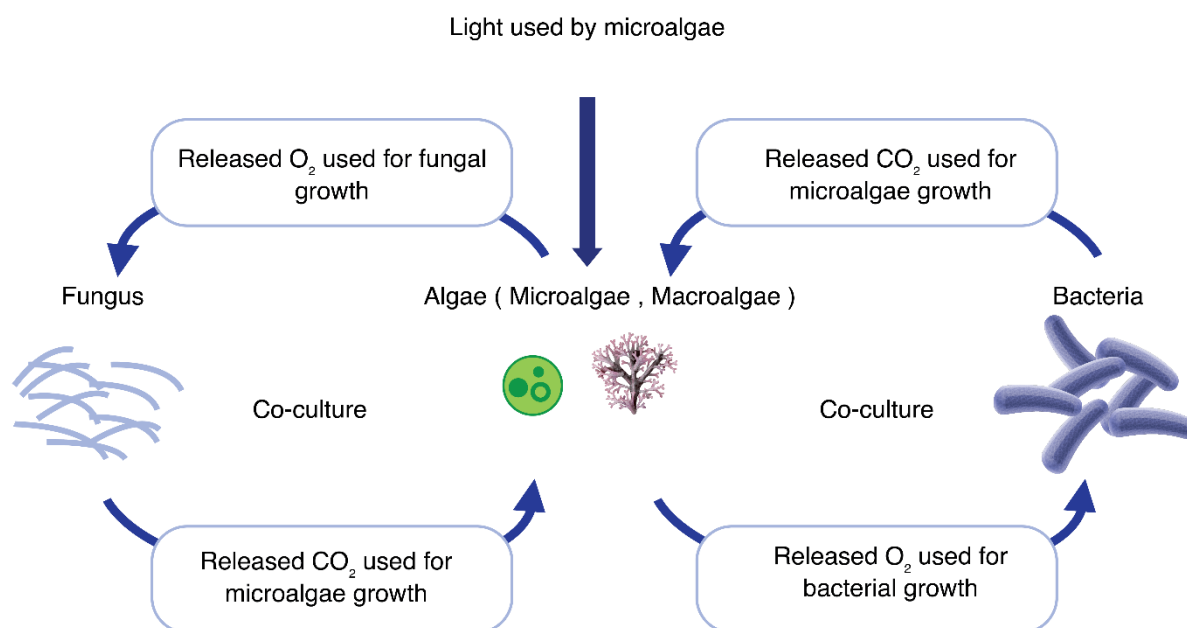


Figure 5. Schematic representation of the algal-fungal co- cultivation and Mechanism of algal-bacterial symbiotic relationship for industrial wastewater treatment.

Removal of contaminants by Consortia of microalgae and macroalgae with bacteria and fungi for wastewater treatment

Nitrogen Removal

The performance of the nitrogen removal process in biological wastewater treatment systems depends on a balance between the nitrification–denitrification stages, ion fluxes within the system, and the uptake of nutrients by microbial and algal biomass. These functional differences account for the variability in performance among different treatment systems. Microalgae–bacteria consortia are usually those that achieve the highest and most stable nitrogen removal efficiencies, as they combine autotrophic oxygen production with bacterial nitrogen transformation metabolisms.

In an inverted fluidised bed biofilm reactor (IFBBR) inoculated not only with *Chlorella* but also with different microalgae from the order *Trebouxiophyceae*, and with bacteria included in the genera *Nitrosomonas*, *Gordonia*, *Ignavibacterium*, and *Limnobacter*, total nitrogen removal exceeded 95% and was maintained for 240 days, without any need for aeration or the addition of an external carbon source. The efficiency of nitrogen removal largely depends on how the consortium manages to carry out complete nitrification and denitrification processes. Mechanistically, this reflects the long-term stability of the system [53].

The same trend was observed with a consortium composed of *Chlorella protothecoides*, *Bacillus licheniformis*, and *Pseudomonas putida*; a system that was operated under blue light conditions (1.8 L min^{-1} , 20% inoculum) and was able to remove 93.7% of the total nitrogen and 98.4% of the ammonium in 48 hours [54]. The rapid removal of ammonium shows that microalgal assimilation and bacterial nitrification operate effectively in tandem. Moreover, the presence of heterotrophic bacteria may have helped to mineralise more organic nitrogen in a short time, which would provide more ammonium than the system would have contained if it consisted only of microalgae and nitrifying bacteria.

In contrast, microalgae–fungi consortia achieved lower nitrogen removal efficiencies, ranging from 74% to 87% [55,13]. Such a difference is consistent with mechanistic expectations. Fungi lack the nitrification pathway, and nitrogen removal in these systems depends primarily on assimilation. Although granule formation improves biomass retention and sedimentation, it does not by itself provide the biochemical pathways needed for complete denitrification. As a result, nitrogen transformation remains, to a large extent, incomplete, especially in wastewater that is high in inorganic nitrogen. This is one reason why the study of the treatment of oil-industry produced water showed that nitrate removal efficiencies were 63.4% for 25 mg L^{-1} , 44.4% for 50 mg L^{-1} , and as low as 31.7% for 100 mg L^{-1} , strongly suggesting a decrease in removal efficiency with increasing initial nitrate concentration [56].

Although macroalgae–bacteria consortia operated in tank-type reactors (20 L tanks) have shown removal efficiencies for total nitrogen (TN), NO_3^- -N, and NO_2^- -N exceeding 99%, which is extremely high, the treatment, which lasted 16 days, revealed that the addition of *Bacillus subtilis* and *Bacillus licheniformis* significantly altered the structure of the bacterial community in both the water and on the algal surface. As a result, functional bacteria responsible for the nitrogen cycle, such as *Algoriphagus* and *Marivita*, were enriched. This interaction between algae and bacteria resulted in a significant enhancement of nitrogenous nutrient removal [57].

A 20 L Moving Bed Biofilm Reactor (MBBR) was used in another experiment to treat wastewater from a marine aquaculture system using the macroalga *Chaetomorpha maxima* directly integrated into the reactor (MBBR–MA). Bacterial biofilm formed on the mobile carriers (K1), whereas the algal filaments existed freely in the medium, taking advantage of continuous aeration and a 12 h light / 12 h dark photoperiod. On average, this co-system achieved a TN reduction of $42.8 \pm 5.5\%$ [5].

Macroalgae–fungi systems are still very much a poorly understood area when it comes to their effectiveness in removing nitrogen. We simply lack sufficiently detailed data on nitrogen speciation

to fully understand the processes occurring in these systems, which represents a major knowledge gap. On the whole, bacteria have to be part of the picture if nitrogen is to undergo a full cycle of transformations. Consortia of microalgae and bacteria are the best solution, as they not only remove nitrogen very quickly but also efficiently, whereas macroalgae–bacteria systems rank next, mainly being able to remove nitrogen by assimilation. On the other hand, metabolic pathways are the limiting factors for microalgae–fungi consortia.

Phosphorus Removal

Phosphorus elimination by algae–microorganism consortia is mainly achieved through assimilation, although it can be further enhanced by interactions that trigger precipitation processes and polyphosphate accumulation. Recent research has developed a vertical rotating biofilm reactor in which a microalgae–bacteria consortium grows as a biofilm on vertically oriented discs that are only partly immersed, so they are alternately in contact with wastewater and air. The system was employed in sequential batch and semi-continuous modes using a mixed effluent composed of municipal wastewater and soybean soaking water. In batch mode, the system was able to remove phosphorus with an efficiency of 97.6% [58].

The high efficiency of this system can be explained by the fact that algal growth is facilitated through the bacterial mineralisation of organic phosphorus compounds. Bacterial activity increases phosphorus availability, whereas the dense biofilm structure helps increase retention time and enhance nutrient capture. In addition, microalgae can store phosphorus in their cells as polyphosphates, which is one of the ways to temporarily store excess nutrients under conditions of high nutrient availability. In a highly specialised agro-industrial setting, the treatment of swine farming wastewater using a combination of microalgae and bacteria has been highly efficient, achieving an approximate 70% reduction in orthophosphate [13].

Regarding microalgae–fungi consortia, two separate studies examined the co-culture of *Tetrademus obliquus* and *Cunninghamella echinulata*, but reported very different phosphorus removal rates. In research on the treatment of oil-produced water, the microalgae–fungi consortium achieved an average phosphorus removal of about 36% at an initial concentration of approximately 30 mg L⁻¹ [56]. By contrast, in research dealing with the tertiary treatment of cheese whey, the same consortium achieved a much higher removal efficiency of between 66% and 70% for lower initial phosphorus concentrations (4.5–18 mg L⁻¹) [55].

This contrast is therefore unlikely to be related to the species employed, but rather to the type of effluent and operational conditions. In addition, oil-produced water has high salinity, a high hydrocarbon load, and a toxic environment that can limit biological phosphorus assimilation and microalgal growth. In contrast, cheese whey contains a simple organic substrate, facilitating biomass development, and thus phosphorus is more readily incorporated into cells. Hence, although the same microorganisms are studied, differences in phosphorus bioavailability, chemical complexity of the medium, and operational parameters explain the variation in removal efficiencies.

Although there are very few studies that explicitly investigate macroalgae–fungi consortia, research on macroalgae consortia with bacteria already shows great potential for phosphorus removal in wastewater. For instance, one experiment demonstrated up to 83.7% phosphorus removal in a Moving Bed Biofilm Reactor (MBBR) via the direct addition of the macroalga *Chaetomorpha maxima* to the reactor (MBBR–MA). In such a system, the bacterial biofilm formed on the mobile carriers (K1), whereas the algal filaments floated freely. This research clearly shows that macroalgae can take up and use soluble nutrients even under real wastewater conditions [5].

Although this level of efficiency is inferior to that seen in some microalgae–bacteria systems, it nevertheless opens the door to considering fungal integration as a means to enhance biosorption and enzymatic phosphorus degradation in future macroalgae–fungi consortia. Microalgae–bacteria and microalgae–fungi consortia offer rapid and efficient phosphorus removal, whereas macroalgae–bacteria combinations are limited by their lower cell division rate and available surface area, although they still have the potential for biomass conversion.

COD Removal

COD removal efficiency is largely dependent on oxygen levels and the ability of heterotrophic organisms to degrade organic matter. Systems that combine microalgae and bacteria have shown COD removal results of between 77% [13] and 95.6% [58], the main reason being that microalgae perform photosynthesis and release oxygen, which helps bacteria carry out aerobic oxidation of organic matter. Such oxygen supply through photosynthesis drastically limits the need for external aeration, resulting in much lower energy consumption. In addition, a stable biofilm provides better microbial retention and prevents biomass loss in semi-continuous setups.

Microalgae–fungi consortia have also been shown to perform effectively, achieving COD removal rates of 75–77% [55]. In microalgae–bacteria systems for dairy wastewater, similar levels of COD

removal were observed [13]; nevertheless, it is not appropriate to interpret these results as indicating equal system effectiveness when applied to swine effluents. Not only the composition but also the organic complexity of the two wastewater types varies considerably: dairy effluents normally contain substrates that are more easily biodegradable, whereas swine wastewater is usually characterised by high levels of organic matter, nitrogen, and more diverse organic fractions. Furthermore, the fungal system reached the COD removal target within three days, whereas the bacterial consortium required approximately six weeks to achieve the same objective. This marked difference in treatment duration highlights fundamentally distinct degradation kinetics.

Macroalgae–fungi systems have achieved up to 80% COD removal using mixed biomass of *Ulva* and *Aspergillus niger*, and 100% with consortia composed of *Laminaria* and *Aspergillus niger* in fish processing wastewater [12]. This is because biosorption by macroalgae complements fungal enzymatic degradation. Nevertheless, oxygen transfer limitations in dense macroalgal biomass may reduce degradation rates compared to those in microalgae.

In macroalgae–bacteria systems used in aquaculture wastewater, COD removal is usually less efficient than in microalgae–bacteria or microalgae–fungi consortia. For example, a recent paper has shown that COD reductions ranged between 50.88% and 68.61% in shrimp farming effluents using *Caulerpa* sp. associated with immobilised bacteria [59].

To sum up, microalgae–bacteria and microalgae–fungi consortia provide faster and more extensive degradation of organic matter, whereas macroalgae–bacteria systems are still mainly based on assimilation processes.

Heavy Metals and Emerging Contaminants

Heavy metals refer to elements with atomic densities of at least 4000 kg m^{-3} [60]. They are a global environmental concern because of their non-biodegradability, toxic effects, and capacity to strongly bioaccumulate. In addition, heavy metals, even at trace levels, may lead to serious human and animal health hazards. Three main categories of heavy metals are commonly recognised: radionuclides (U, Ra, Am, and Th), precious metals (Au, Pd, Pt, and Ru), and toxic metals (Cu, Cr, As, Zn, Ni, Ag, Sn, Co, and Pb) [61,62].

Heavy metals in water are not only the result of natural geological activities but also of human-induced factors such as industrial waste discharges and agricultural runoff. Accordingly, a large number of techniques have been proposed to eliminate heavy metals from polluted waters, but their effectiveness differs. Among these approaches, biological treatments have come to be seen as a promising option. For instance, a combination of microalgae and bacteria can remove up to 84% of Fe, 44% of Cu, and 48% of Mn from pig farm wastewater [13].

It is believed that metal binding occurs through functional groups on the cell walls of algae and bacteria, which carry negative charges, such as carboxyl, hydroxyl, and phosphate groups. Nevertheless, copper and manganese were only partially removed, indicating differences in metal-specific affinity. These systems may also respond more effectively under simultaneous stress conditions, a feature observed in microalgae–fungi co-culture systems. A co-culture of *Chlorella sorokiniana* and *Aspergillus oryzae* resulted in the removal of 86% of Zn^{2+} and the degradation of 84.6% of estrone [58].

Moreover, metabolite profiling showed an upregulation of the tricarboxylic acid cycle and an increased release of organic acids, which may be responsible for metal chelation and oxidative degradation of endocrine disruptors, respectively. Thus, this reflects a metabolic acclimation during stress exposure rather than mere passive adsorption.

Recent studies indicate that integrated macroalgae–fungi systems represent an effective and eco-friendly approach to wastewater treatment, as they rely on the synergistic effects of nutrient biosorption and fungal enzymatic activity. For example, a combination of the macroalga *Spirogyra maxima* and the fungi *Trichoderma asperellum* or *Mucor racemosus* was employed to treat Wadi al Kharazi wastewater in Iraq: the co-culture reduced bicarbonate concentrations from 204.9 to 162.8 mg L^{-1} and decreased sulphate levels by up to 48%, thus demonstrating that the combined effect of the two organisms is superior to that of either one used individually [63].

In addition, macroalgae–fungi assemblies are useful for the remediation of pharmaceutical residues, achieving biosorption levels above 70% and enzymatic breakdown of persistent organic compounds through enzymes such as laccases and peroxidases [64,65]. Microalgae–fungi consortia are the most effective for complex contaminants and metals, followed by microalgae–bacteria systems. Macroalgae–bacteria systems still require optimisation and long-term studies. Nevertheless, issues such as metal desorption, biomass recovery strategies, and the long-term stability of these systems remain insufficiently explored.

Challenges

Despite the promising potential of algal–microorganism consortia for wastewater treatment, several scientific, technical, and operational barriers still limit their large-scale implementation. One of the main challenges lies in selecting compatible species and controlling interspecies interactions. The performance of these systems strongly depends on the metabolic cooperation between microalgae, macroalgae, bacteria, and fungi. These interactions are highly sensitive to environmental and operational parameters, including light intensity, pH, temperature, mixing conditions, and nutrient availability. Inadequate control of these parameters can disrupt consortium stability, reduce biomass productivity, and ultimately decrease pollutant removal efficiency. Another important limitation concerns the use of filamentous fungi in algal–fungal systems. Although many fungal species exhibit excellent bioflocculation capacity, some are opportunistic or potentially pathogenic microorganisms, raising biosafety concerns and limiting their application in large-scale wastewater treatment processes. Consequently, ensuring the safe use of fungal strains remains a critical issue for the development of these systems.

In addition, most studies investigating algal–microorganism consortia have been conducted under controlled laboratory conditions, often using sterilised or synthetic wastewater. In contrast, real wastewater environments contain complex indigenous microbial communities, variable pollutant loads, and fluctuating environmental conditions. These factors may significantly influence consortium stability, microbial competition, and overall treatment efficiency, making the transition from laboratory studies to real wastewater systems particularly challenging.

From a technical standpoint, reactor design and operational control also pose significant challenges. It remains technically difficult to achieve adequate light penetration in these systems, efficient biomass distribution, and stable mixing conditions in large-scale systems remain challenging.

Furthermore, the need for optimised lighting, mixing, and process control may increase operational costs, thereby limiting the economic feasibility of large-scale applications.

Finally, beyond these technical challenges, there are also issues related to regulation and public acceptance. The use of bio-based treatment technologies involving diverse microbial biomass may require specific biosafety regulations and public support, particularly when applied to domestic or industrial wastewater treatment systems.

Future Perspectives and Conclusion

Algae–microorganism consortia such as microalgae–bacteria, microalgae–fungi, macroalgae–bacteria, and macroalgae–fungi represent some of the most promising biotechnological approaches for sustainable wastewater treatment. Various studies have demonstrated the ability of these consortia to efficiently remove nitrogen, phosphorus, organic matter (COD), certain heavy metals, and emerging contaminants, together with reduced energy consumption associated with aeration and the potential for biomass valorisation. The combined metabolic activities of these organisms not only result in superior treatment efficiency compared to single-species cultures but also pave the way for developing less energy-demanding processes.

In addition to providing an overview of the salient features of the different types of algae–microorganism consortia used in wastewater treatment, this paper also outlines the major biochemical processes through which metabolic synergies lead to higher pollutant removal efficiencies than in single-species systems. A further contribution of this paper is the identification of principal research gaps, including the scarcity of work on macroalgae-based systems and on algae–fungi interactions. Among the technical and scientific issues still to be addressed are the stability of consortia over long periods, microbial community dynamics, biosafety considerations (especially concerning fungal strains), pilot-scale validation, and the application of advances in reactor design. Moreover, economic evaluations and life cycle analyses are still underrepresented in the literature.

Future research directions could include the identification of robust and non-pathogenic algal and fungal species capable of thriving under natural environmental conditions and withstanding environmental stress, including strains with favourable growth characteristics and high productivity. Further work should focus on improving co-culture systems so that they operate effectively under practical conditions, including natural light and minimal external nutrient supply. The development of integrated systems that promote biomass retention and enhance operational

stability is also required, alongside pilot-scale experiments to assess long-term performance and economic viability.

In sum, although algae–microorganism-based wastewater treatment technologies show considerable promise, their progression to industrial-scale applications will require systematic comparative studies, advances in reactor engineering, and rigorous techno-economic evaluation.

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Optimizing the Diagnosis of Tuberculous Pericarditis through the Use of GeneXpert MTB/RIF

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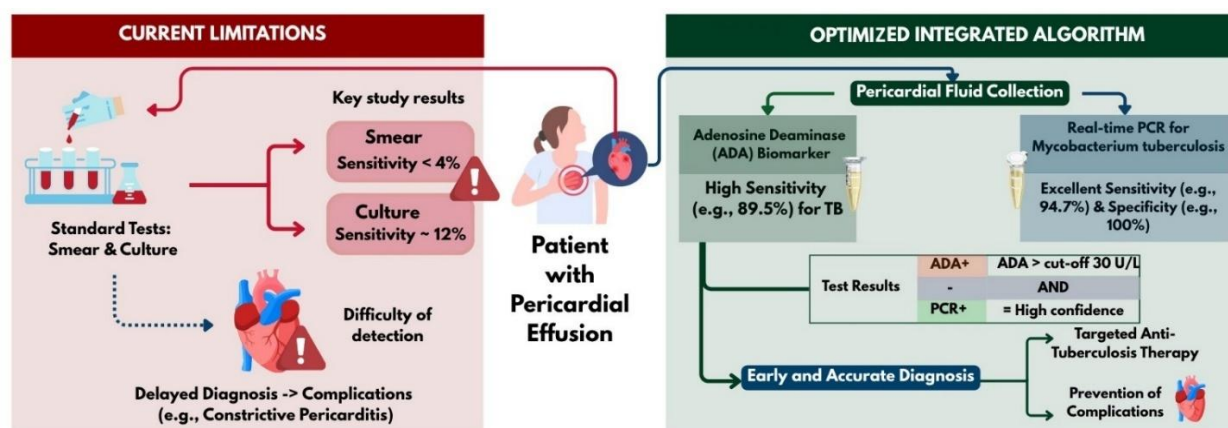
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Graphical abstract

This prospective study evaluated the GeneXpert MTB/RIF® test (Cepheid Inc., USA) for diagnosing pericardial tuberculosis (TBP) in comparison with culture, the reference method recommended by the Moroccan National Tuberculosis Control Program (PNLAT). Twenty-seven pericardial fluid samples collected over 36 months at Ibn Sina University Hospital, Rabat, were analysed using bacilloscopy, culture, and GeneXpert MTB/RIF. Microscopy showed no positive results, whereas GeneXpert MTB/RIF and culture yielded positivity rates of 14.81% and 11.11%, respectively; the assay also demonstrated 100% sensitivity and NPV, with 95.8% specificity and 75% PPV. These findings indicate that GeneXpert MTB/RIF is a rapid and reliable first-line diagnostic tool for pericardial tuberculosis, providing results much faster than culture.



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Abstract: This study evaluated the performance of the GeneXpert MTB/RIF® test (Cepheid Inc., USA) for detecting *Mycobacterium tuberculosis* and Rifampicin resistance in pericardial tuberculosis (TBP), compared to culture, the reference method recommended by the Moroccan National Tuberculosis Control Programme (PNLAT). Conducted prospectively over 36 months, the study analysed 27 pericardial fluid samples from suspected TBP cases at Ibn Sina University Hospital, Rabat. Conventional methods (bacilloscopy and culture) were compared to GeneXpert MTB/RIF. Microscopy showed no positive results, whereas GeneXpert MTB/RIF and culture yielded positivity rates of 14.81% and 11.11%, respectively. Most culture-positive samples were bacilloscopy-negative. The GeneXpert MTB/RIF assay showed 100% sensitivity and NPV, with 95.8% specificity and 75% PPV. No Rifampicin resistance was identified. The findings suggest that GeneXpert MTB/RIF is a rapid and reliable first-line diagnostic tool for pericardial tuberculosis and may replace smear microscopy and culture, which require prolonged incubation.

Keywords: Pericardial Tuberculosis, GeneXpert MTB/RIF, Molecular Diagnosis.

Introduction

Tuberculosis (TB) continues to represent a major global health concern, particularly in low- and middle-income countries [1]. In regions where the burden of both tuberculosis and human immunodeficiency virus (HIV) is high, mortality associated with TB remains alarmingly elevated, especially among individuals co-infected with HIV/AIDS [2].

In immunocompromised patients, this high mortality is largely attributed to the increased occurrence of disseminated and severe forms of extrapulmonary TB, such as tuberculous pericarditis (PTB) [3]. PTB is the most lethal manifestation of extrapulmonary TB. This form of TB is the leading cause of clinically significant pericardial effusion in TB-endemic developing countries, accounting for up to 90% of pericardial effusions in HIV-infected individuals and 50–70% in HIV-uninfected individuals [4].

Mortality rates from tuberculous pericarditis increase from 17% to 40% within six months after diagnosis [3], and PTB accounts for approximately 7% of hospital admissions for acute heart failure in Africa [5]. The incidence of PTB has progressively increased [6], and it is the most common cause of pericarditis in areas with a high incidence of tuberculosis [6,7]. Early and accurate diagnostic measures are essential for controlling fatal extrapulmonary tuberculous infections [8].

Despite this disease burden and associated mortality, the diagnosis of PTB remains difficult, mainly because of the lack of simple, rapid, accessible, and accurate diagnostic tests [3]. This difficulty arises from the fact that the amount of MTB in pericardial fluid is generally very low. This biological sample is usually paucibacillary, with diagnostic accuracy by culture and microscopic smear estimated at 50% and 5%, respectively [4].

In addition, MTB culture requires several weeks to provide results [9]. Consequently, the definitive diagnosis of PTB remains complex and is often delayed [10].

Recent studies have suggested that rapid initiation of anti-tuberculosis treatment may reduce mortality, making the search for new rapid diagnostic tests for PTB crucial [11]. To improve the prognosis of PTB, early diagnosis and antibacterial treatment are essential. Early diagnosis of PTB nevertheless remains difficult in many suspected cases [7]. Without prompt and effective treatment, PTB can lead to serious complications, such as constrictive pericarditis and even death [7].

Until 2018, Lowenstein-Jensen (LJ) culture was considered the gold standard for diagnosing tuberculous pericarditis, as early non-invasive diagnostic procedures, such as chest radiographs, revealed changes suggestive of tuberculosis in only 30% of cases, and echocardiography showed only a large sling-like projection of pericardial effusion, which is too nonspecific to indicate a

tuberculous aetiology [12,13]. Nevertheless, LJ culture is time-consuming and complex. It is therefore not recommended for routine use [14].

Other alternatives, such as histopathological examination, require invasive procedures and expertise available only in a limited number of medical centres. In addition, its sensitivity remains highly variable, ranging from 30% to 70% [15]. Histopathological examination is therefore not recommended as a routine test [16]. Currently, the Xpert MTB/RIF test is FDA-approved as a diagnostic test for pulmonary tuberculosis. This test detects members of the *Mycobacterium tuberculosis* complex and rifampicin resistance (RR) through nucleic acid amplification. It has proven useful in diagnosing pulmonary tuberculosis, with a sensitivity of 89% and a specificity of 99% [15].

The Xpert MTB/RIF test is a recent quantitative PCR assay that allows rapid diagnosis of *Mycobacterium tuberculosis* (MTB) and rifampicin resistance (RR), with results available in less than two hours [17]. WHO guidelines for the integration of the GeneXpert MTB/RIF test into the TB diagnostic process are based on its rapid results and proven performance, particularly its sensitivity and specificity for the diagnosis of both pulmonary and extrapulmonary TB [18].

The use of rapid molecular techniques for the diagnosis of tuberculosis is recognized by the World Health Organization as an essential element in tuberculosis control strategies, both for initial diagnosis and for monitoring disease progression [19].

Considering the good performance of Xpert MTB/RIF in the diagnosis of tuberculosis, the World Health Organization has recommended its use for the early diagnosis of tuberculosis since 2010 [20]. Xpert MTB/RIF is also applicable to extrapulmonary tuberculosis (EPTB), such as lymph node tuberculosis, and has demonstrated excellent diagnostic efficiency [21]. The application of Xpert MTB/RIF in the diagnosis of PTB offers unique advantages.

The objective of our study was to evaluate the performance of GeneXpert MTB/RIF in the diagnosis of pericardial tuberculosis (PTB) in a tuberculosis-endemic country, in order to assess the sensitivity and specificity of this molecular technique compared to culture, which constitutes the reference method (gold standard).

Patients and Methods

This retrospective study covered a period of 36 months and concerned pericardial fluid samples (n=27) collected between January 2020 and January 2023 from patients suspected of pericardial tuberculosis in the Cardiology Department of Ibn Sina University Hospital. These samples were received at the Bacteriology Laboratory of Ibn Sina University Hospital for the detection of MTB

using conventional methods (bacilloscopy and culture), as well as the molecular method using the GeneXpert MTB/RIF test.

Samples were cultured on Lowenstein-Jensen media and incubated at 37°C, with daily observation during the first week and twice-weekly observation for up to 8 weeks [22]. Smear microscopy was performed according to the Ziehl–Neelsen method, as recommended by the World Health Organization [23]. The GeneXpert assay was performed according to the manufacturer's Cepheid protocol (2009), allowing rapid detection of *M. tuberculosis* DNA and rifampicin resistance mutations in less than 120 minutes [24,17].

Results

Distribution of tuberculosis patients by age and sex

In this study, 27 patient samples were analysed, of which 4 were found to be positive using GeneXpert MTB/RIF, corresponding to a positivity rate of 14.81%. The mean age of tuberculosis patients was 29.75 ± 14.43 years, with ages ranging from 14 to 48 years. A male predominance was observed, representing 75% of cases, corresponding to 3 out of 4 patients (Table 1).

Table 1. Distribution of patients with PTB revealed by GX according to sex

Sex	Number of Patients (n)	Percentage (%)
Female	1	25
Male	3	75
Total	4	100
Male/Female Ratio	03:01	

Distribution of patients with PTB according to GeneXpert MTB/RIF detection levels

The results of this study on the molecular detection of pericardial tuberculosis (PTB) by GeneXpert MTB/RIF reveal that the majority of cases, representing 75% of patients, were identified at a low level (n=0), and only one case was detected at a medium level. This finding is explained by the very nature of this mycobacterial infection, which is often characterized by paucibacillary samples and a low bacillary load in pericardial specimens.

This particularity makes detection more complex and requires the use of sensitive techniques capable of identifying the infection even at low detection levels. These results therefore underline the importance of using appropriate and sensitive diagnostic methods for more accurate detection of this form of extrapulmonary tuberculosis

Comparison of GeneXpert MTB/RIF results versus Bacilloscopy and Culture

The positivity rates for microscopy, GeneXpert MTB/RIF, and culture were 0% (n=0), 14.81% (n=4), and 11.11% (n=3), respectively. Comparison of GeneXpert results with those of smear microscopy and culture revealed notable discrepancies in the diagnosis of pericardial tuberculosis.

Indeed, all samples were negative by smear microscopy, whereas 75% of samples were culture-positive but smear-negative, implying that the clinician would have had to wait several weeks for culture results.

Furthermore, only one specimen (25%) showed negative results for both culture and smear microscopy, suggesting that a physician could potentially miss a diagnosis of pericardial tuberculosis if relying solely on negative conventional laboratory tests, thus making early identification of this infection difficult. These findings highlight the importance of using GX, which provides more rapid and sensitive detection, particularly in cases where both smear microscopy and culture are negative.

Performance of GX in the diagnosis of pericardial tuberculosis (PTB)

The contribution of the GeneXpert MTB/RIF test to the diagnosis of pericardial tuberculosis (PTB) is illustrated by the results obtained in our study, which demonstrate notable performance of the test compared with culture (gold standard). Indeed, the negative predictive value (NPV) was 100%, indicating that all GeneXpert-negative cases were truly free from pericardial tuberculosis.

Sensitivity (Se) also reached 100%, meaning that the test was able to identify all forms of PTB in the analysed samples. Specificity (Sp) was 95.8%, highlighting the test's ability to exclude uninfected cases. The positive predictive value (PPV) was 75%, indicating that a positive test result was associated with a 75% probability that the patient truly had PTB, thus reinforcing the reliability of this diagnostic method.

Finally, the Kappa index of 0.886 reflects good agreement between the results of GeneXpert MTB/RIF and those of the reference test (culture), indicating satisfactory performance in the context of this molecular diagnosis (Table 2).

Rifampicin resistance (RR) cases revealed by GX

The results of rifampicin resistance (RR) testing by GeneXpert MTB/RIF on the analysed pericardial fluid samples revealed no cases of resistance (n=0). All samples showed full susceptibility to rifampicin, confirming the absence of drug-resistant pericardial tuberculosis (RR-PTB).

Table 2. Results of GX Performance in the Diagnosis of Pericardial Tuberculosis Compared to Culture (Gold Standard)

GX in the Diagnosis of PTB	Positivity Rate (%)	RR (%)	NPV (%)	PPV (%)	Se (%)	Sp (%)	Kappa
Our study – Morocco (Rabat)	14.81 (n=4)	0 (n=0)	100	75	100	95.8	0.886

GX: GeneXpert MTB/RIF; **RR:** Rifampicin Resistance; **NPV:** negative predictive value; **PPV:** positive predictive value; **Se:** sensitivity; **Sp:** specificity

Discussion

Pericardial tuberculosis (PTB) accounts for approximately 1–8% of all new TB cases [25]. Similar to extrapulmonary tuberculosis (EPTB), PTB is also paucibacillary, making early diagnosis more difficult [4]. Delayed diagnosis or misdiagnosis leads to more severe clinical outcomes in PTB [3]. Conventional acid-fast bacilli (AFB) tests do not provide sufficiently high diagnostic performance to enable early and rapid diagnosis of PTB. There is therefore an urgent need for early, rapid, and direct detection methods for *Mycobacterium tuberculosis* (MTB) in order to reduce the risk of serious adverse events associated with PTB.

Nucleic acid amplification tests (NAATs), which directly detect nucleic acids from pathogenic bacteria, are widely used for the detection of pathogens such as tuberculosis [26]. NAATs are increasingly valued for their efficiency and accuracy in the early diagnosis of infectious diseases [27]. The Xpert MTB/RIF test is currently the most commonly used NAAT for the diagnosis of tuberculosis. This test detects MTB DNA through an automated real-time polymerase chain reaction and provides results in less than two hours [28].

Xpert MTB/RIF has excellent diagnostic efficacy for pulmonary tuberculosis (PTB) as well as extrapulmonary tuberculosis (EPTB). The World Health Organization also recommends the use of Xpert MTB/RIF for the early diagnosis of PTB, tuberculous meningitis, and lymph node tuberculosis [15,29]. This test is therefore used for the early diagnosis of PTB.

According to the national guidelines of the Ministry of Health and Social Protection for the diagnosis and management of extrapulmonary tuberculosis, certain forms of tuberculosis, such as neuromeningeal tuberculosis (TMN) or pericardial tuberculosis (PTB), may have serious consequences and compromise the patient’s prognosis. These forms require early detection and appropriate treatment in order to prevent severe complications and improve the chances of recovery [30].

Frequency of pericardial tuberculosis (PTB)

In this study, the positivity rate for pericardial tuberculosis (PTB) using the GeneXpert MTB/RIF (GX) test was 14.81%. This result lies within the lower range of rates reported in the literature, although it remains higher than those described in previous studies. For example, the studies by Diallo *et al.* [31] and Ullah *et al.* [41] reported no PTB positivity by GX (0%), which may be explained by differences in the study populations or in the diagnostic methods used [46] (Table 3).

Other studies have reported lower positivity rates, such as those by Saeed *et al.* [36] and Allahyartorkaman *et al.* [39], with rates of 10.16% and 10.83%, respectively. Slightly higher rates were observed in studies such as that of Mahmood *et al.* [43], which reported a rate of 11.30%. In comparison, studies by Toure *et al.* [32] and Khan *et al.* [38] reported higher positivity rates, reaching 20% and 20.69%, respectively. At the upper extreme, some recent studies reported much higher positivity rates, such as those by Yu *et al.* [42,45], which found rates of 47.37% and 61.11%, respectively [46] (Table 3).

These results suggest that the diagnostic performance of GeneXpert may vary according to the specific characteristics of the study population, the diagnostic criteria, and the methodologies employed. Our study nevertheless shows a trend similar to that reported in more recent studies, such as that of Xu *et al.* [35], which reported a rate of 25.71%, highlighting a general tendency towards improved PTB detection by GeneXpert. These differences in positivity rates may also be influenced by the evolution of diagnostic protocols and improvements in biological sampling and MTB detection technologies [46] (Figure 1).

Despite intensive efforts by the National Tuberculosis Control Programme (PNLAT) in Morocco, which have led to improvements in disease management and control, TB cases continue to be reported, particularly extrapulmonary tuberculosis (EPT), the frequency of which is steadily increasing. This worrying trend also affects pericardial tuberculosis (PTB), the incidence of which appears to be rising, especially among patients *without associated pulmonary tuberculosis*. This situation considerably complicates the laboratory diagnosis of PTB, as the absence of obvious pulmonary signs makes early identification of the disease more difficult (Figures 2, 3 and 4).

Table 3. Comparative Evaluation of GeneXpert MTB/RIF in the Diagnosis of Pericardial Tuberculosis (PTB) [31-45].

N°	Author Name	Publication Year	City/Country	n	Positive Samples	RR	NPV (%)	PPV (%)	Se (%)	Sp (%)
—	Our study	2025	Morocco (Rabat)	27	14.81% (n=4)	0% (n=0)	100	75	100	95.8
1	Diallo <i>et al.</i> [31]	2022	Senegal (Ziguinchor)	3	0% (n=0)	—	—	—	—	—
2	Toure <i>et al.</i> [32]	2017	Senegal (Dakar)	5	20% (n=1)	—	—	—	—	—
3	Pandie <i>et al.</i> [33]	2014	South Africa (Cape Town)	13	—	—	86.6	100	63.8	100
4	Yu <i>et al.</i> [34]	2017	China (Zhejiang)	27	44.44% (n=12)	0% (n=0)	66.7	100	70.6	100
5	Xu <i>et al.</i> [35]	2020	China (Zhejiang)	105	25.71% (n=27)	14.81% (n=4)	83.3	96.3	66.7	98.5
6	Saeed <i>et al.</i> [36]	2017	Pakistan (Lahore)	128	10.16% (n=13)	23.08% (n=3)	95.6	100	72.2	100
7	Sharma <i>et al.</i> [37]	2014	India (New Delhi)	20	—	—	83	50	25	94
8	Khan <i>et al.</i> [38]	2018	Pakistan (Peshawar)	29	20.69% (n=6)	—	86	100	86	100
9	Allahyartorkaman <i>et al.</i> [39]	2019	Iran (Tehran)	120	10.83% (n=13)	—	97.3	66.6	40	99.1
10	Theron <i>et al.</i> [40]	2014	South Africa (Cape Town)	131	35.11% (n=46)	—	—	—	46	94
11	Ullah <i>et al.</i> [41]	2017	Pakistan (Peshawar)	12	0% (n=0)	—	100	100	100	100
12	Yu <i>et al.</i> [42]	2022	China (Zhejiang)	18	61.11% (n=11)	—	87.9	68.8	61.1	91.1
13	Mahmood <i>et al.</i> [43]	2018	Pakistan (Sheikhupura)	115	11.30% (n=13)	—	95.6	100	72.2	100
14	Gopalaswamy <i>et al.</i> [44]	2021	India (Tamil Nadu)	—	—	—	—	—	60	88
15	Yu <i>et al.</i> [45]	2020	China (Zhejiang)	19	47.37% (n=9)	—	—	—	64	100

Note: PTB = pericardial tuberculosis; *n* = number of samples analysed, RR = rifampicin resistance; NPV = negative predictive value; PPV = positive predictive value; Se = sensitivity; Sp = specificity.

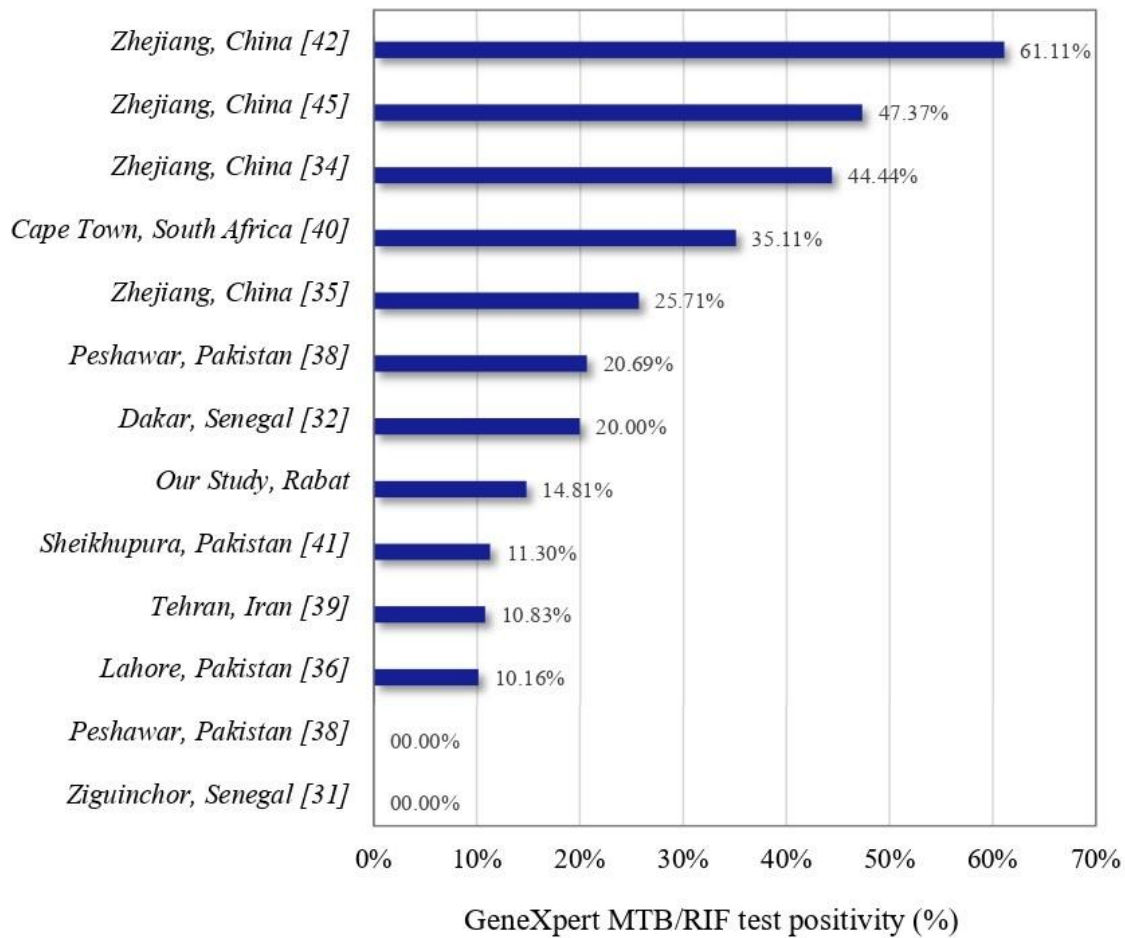


Figure 1. Comparison of GeneXpert MTB/RIF positivity rates for PTB diagnosis across different countries (Table 3)

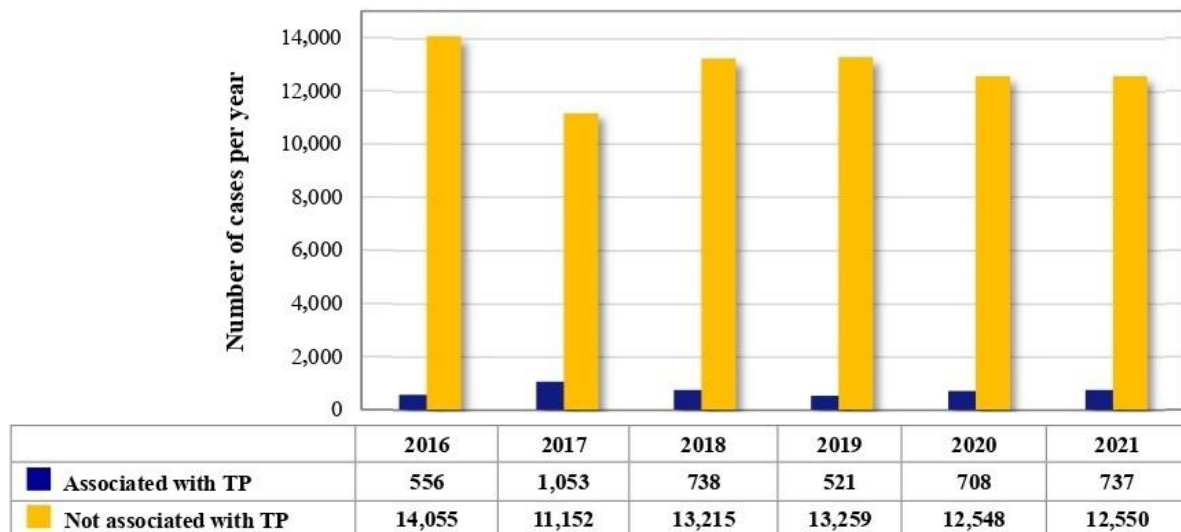


Figure 2. Number of cases of Extra-pulmonary Tuberculosis (EPT) in Morocco from 2016 to 2021 [47]

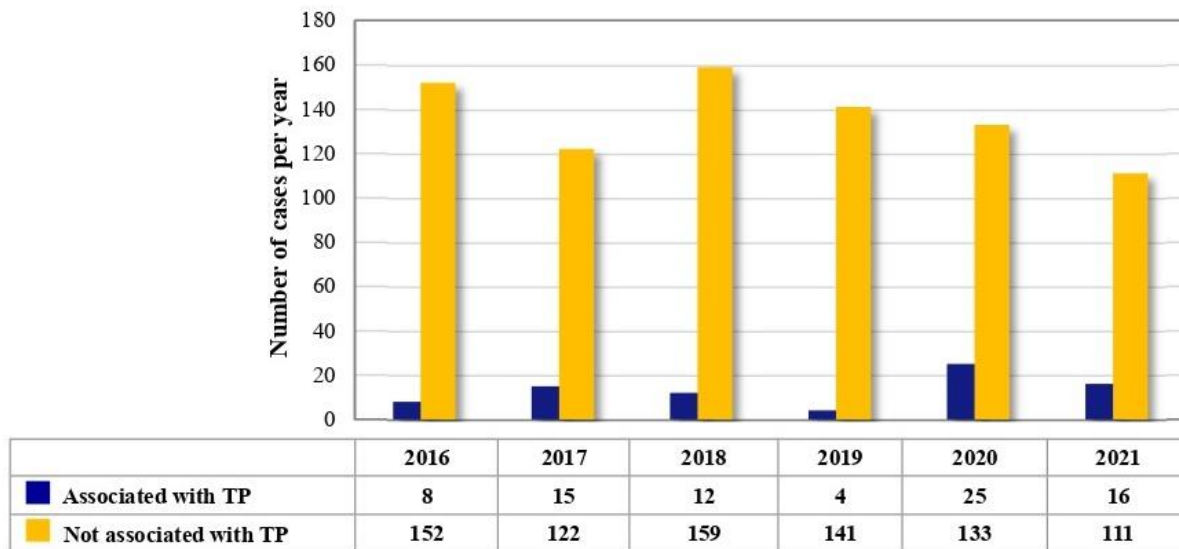


Figure 3. Number of cases of tuberculous pericarditis associated and not associated with tuberculosis Pulmonary disease in Morocco from 2016 to 2021 [47].

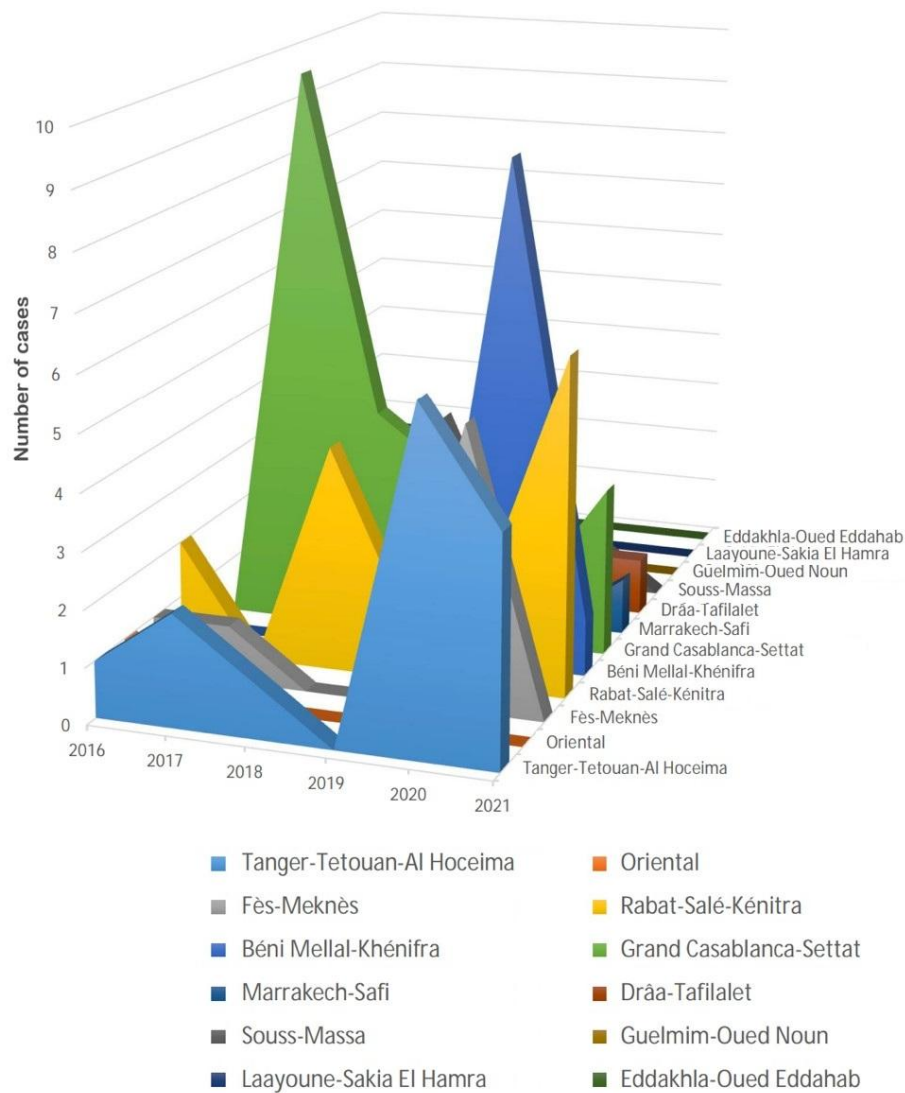


Figure 4. Trend profiles of tuberculous pericarditis cases by region from 2016 to 2021 [47]

Age and Sex of the Population with PTB

In our study, the mean age of patients with tuberculosis was 29.75 ± 14.43 years, with ages ranging from 14 to 48 years. A male predominance was observed, with men representing 75% of the study population, corresponding to three out of four patients. These results are consistent with the literature, which emphasizes that age and sex have biological and social effects that are often difficult to distinguish. Although the risk of developing primary disease is lower in children than in adults, adults are more likely to present severe forms affecting organs other than the lungs, such as tuberculous meningitis and pericardial tuberculosis [48].

According to the World Health Organization, the higher proportion of TB cases among men is consistent with data from national TB prevalence surveys, which indicate that the disease affects more men than women, with greater gaps in case detection and reporting among men. These findings are in agreement with our results, which also reveal a male predominance in our study population [49].

The Performance of GeneXpert MTB/RIF in the Diagnosis of PTB

Specificity (Sp)

In our study, the specificity of GX in the diagnosis of pericardial tuberculosis (95.80%) falls within the range of results reported in the literature. Similar studies, such as those by Gopalaswamy *et al.* [44] (88%), Yu *et al.* [42] (91.10%), Sharma *et al.* [37] (94%), and Theron *et al.* [40] (94%), reported slightly lower values, which may be explained by methodological differences or variations in endemicity rates among the populations studied (Table 3).

Other studies, such as those by Hu *et al.* [35] (98.50%), Allahyartorkaman *et al.* [39] (99.10%), as well as those by Pandie *et al.* [33], Yu *et al.* [34], Saeed *et al.* [36], Khan *et al.* [38], Ullah *et al.* [41], Mahmood *et al.* [43], and Yu *et al.* [45], reported a specificity of 100%, reflecting optimal results and probably a more selective population or particularly rigorous diagnostic methodologies. Overall, our result of 95.80% confirms the effectiveness of GX in the diagnosis of PTB and remains within a significant range compared to international studies (Figure 5).

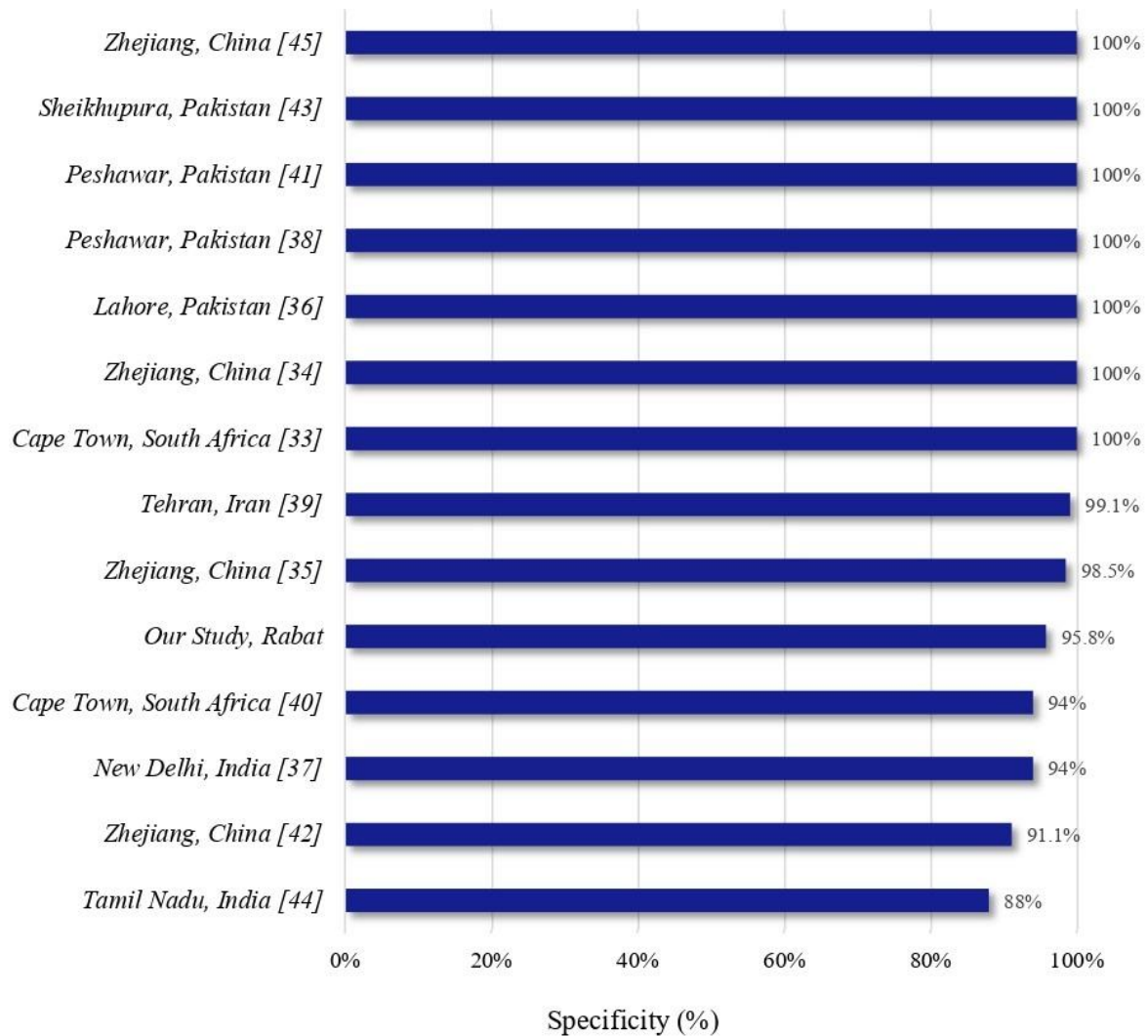


Figure 5. Comparison of GeneXpert MTB/RIF specificity (Sp) in the diagnosis of PTB across different countries (Table 3)

Sensitivity (Se)

In our study, the sensitivity (Se) of GX in diagnosing PTB (100%) is at the upper end of the values reported in the literature and is in perfect agreement with the best performances observed in previous studies. Earlier studies, such as those by Sharma *et al.* [37] (25%), Allahyartorkaman *et al.* [39] (40%), and Theron *et al.* [40] (46%), reported much lower results, reflecting the challenges associated with the early detection of tuberculosis in certain populations. Other studies, such as those by Gopalaswamy *et al.* [44] (60%), Yu *et al.* [42] (61.10%), and Pandie *et al.* [33] (63.80%), reported higher sensitivities, although still lower than that observed in our study [46] (Table 3)

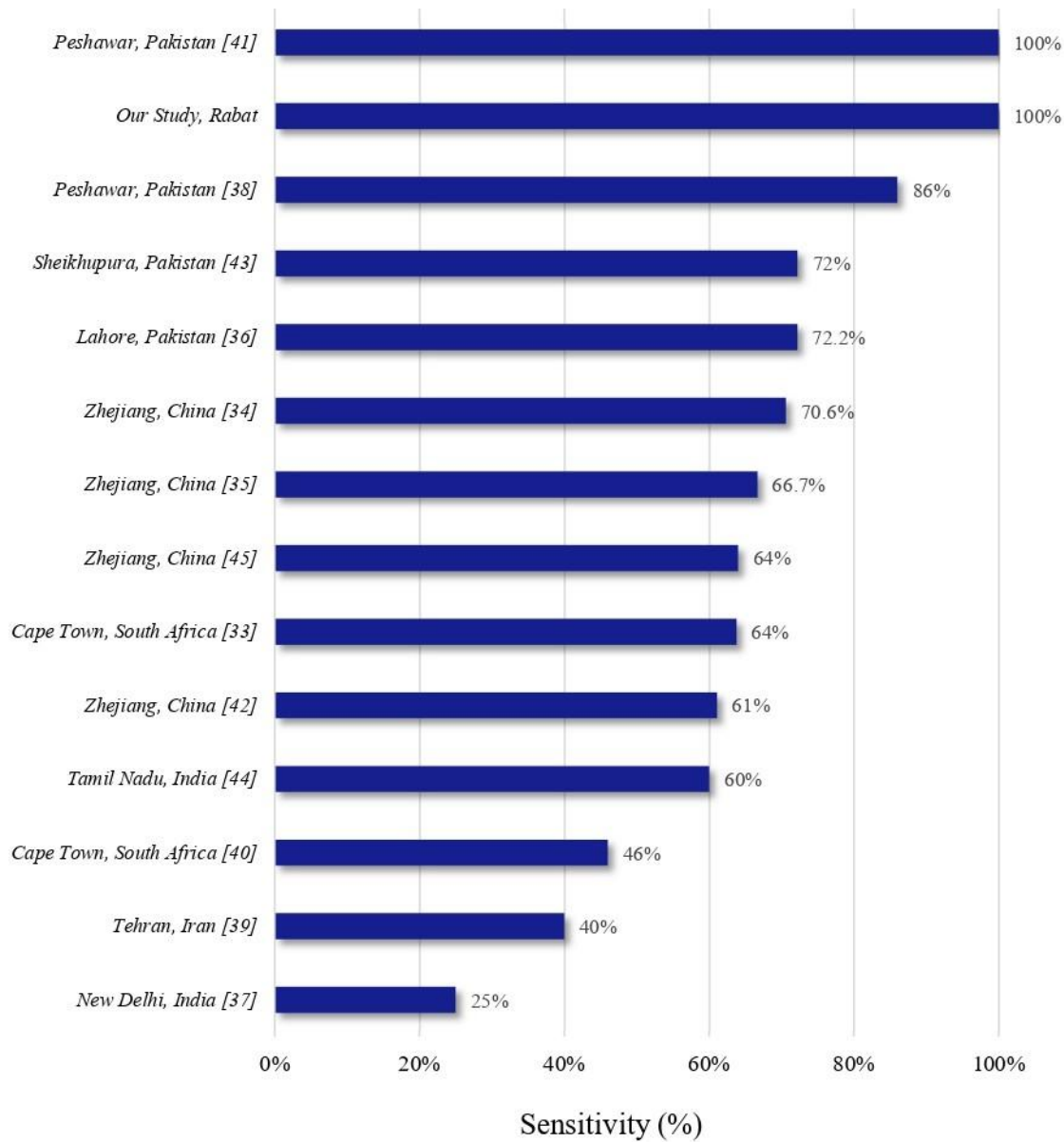


Figure 6. Comparative sensitivity (%) of GeneXpert MTB/RIF for the diagnosis of PTB across different countries (Table 3)

In particular, the studies by Hu *et al.* [35] (66.70%), Yu *et al.* [34] (70.60%), Saeed *et al.* [36] (72.20%), Mahmood *et al.* [43] (72.20%), and Khan *et al.* [38] (86%) demonstrated progressively improved results, with sensitivities increasing over the years alongside technological advances in diagnostic tests. It should be noted that the studies by Ullah *et al.* [41] and our study both reached the maximum sensitivity of 100%, which underlines the effectiveness of GX in the detection of tuberculosis (TB), including severe or atypical forms such as pericardial tuberculosis [46] (Figure 6).

Negative Predictive Value (NPV)

In our study, the negative predictive value (NPV) of GX in the diagnosis of pulmonary tuberculosis (100%) is among the highest reported in the literature and is consistent with the results obtained by Ullah *et al.* [41] (100%). The high NPV observed in our study indicates that GX is particularly effective in excluding the disease in patients without tuberculosis, thereby reducing the risk of false-negative results. Other studies, such as those by Yu *et al.* [34] (66.70%), Sharma *et al.* [38] (83%), and Hu *et al.* [35] (83.30%), reported lower NPVs, which may be related to differences in tuberculosis endemicity among the countries included in these studies [46] (Table 3).

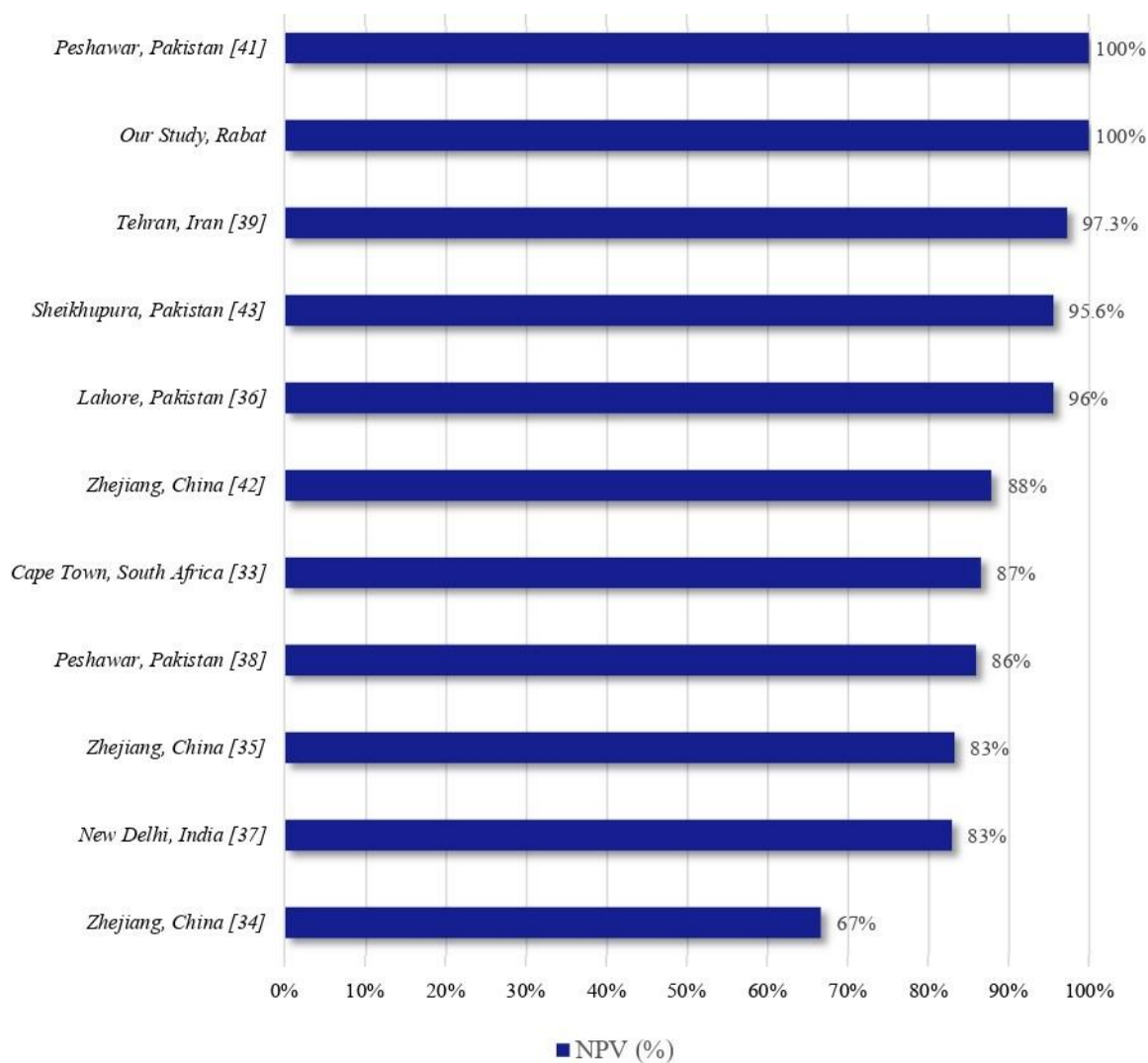


Figure 7. Comparison of GeneXpert MTB/RIF Negative Predictive Value (NPV) in the Diagnosis of PTB in Different Countries (Table 3)

On the other hand, several recent studies, such as those by Khan *et al.* [38] (86%), Pandie *et al.* [33] (86.60%), Yu *et al.* [42] (87.90%), as well as Saeed *et al.* [36] and Mahmood *et al.* [43] (95.60%), reported higher NPVs, although these values remained lower than those observed in our

study. These results reinforce the idea that GX, through its high specificity and sensitivity, offers excellent ability to identify non-TB patients, even in severe and complex forms of the disease such as pericardial TB.

In summary, our study confirms the crucial role of GX in the diagnosis of TB, with an optimal NPV, thereby ensuring reliable exclusion of negative cases [46] (Figure 7).

Predictive Value (PPV)

In our study, the positive predictive value (PPV) of GX in the diagnosis of pericardial tuberculosis (75%) falls within an intermediate range compared with other studies. Although this result is lower than those observed in some high-performing studies, such as Hu *et al.* [35] (96.30%), and several studies in which the PPV reached 100%, including those by Pandie *et al.* [33], Yu *et al.* [34], Saeed *et al.* [36], Khan *et al.* [38], Ullah *et al.* [41], and Mahmood *et al.* [43], it remains significantly higher than the values reported in other studies, such as those by Sharma *et al.* [37] (50%) and Allahyartorkaman *et al.* [39] (66.60%) [46] (Table 3) (Figure 8).

The PPV observed in our study, at 75%, demonstrates that GX is relatively effective in confirming tuberculosis in patients presenting symptoms compatible with the disease, although some variability remains according to the characteristics of the populations studied. Despite this variability, GX remains a powerful diagnostic test with a high PPV, indicating good ability to confirm positive cases, particularly in severe or complex forms of tuberculosis such as pericardial tuberculosis (Table 3).

Rifampicin Resistance (RTP – RR)

In our study, no cases of resistance were detected by the GX test in pericardial tuberculosis cases, which represents a favourable result compared to with other similar studies. Previous studies on pericardial tuberculosis have reported variable resistance rates, often depending on the region and treatment protocols. For example, studies conducted by Hu *et al.* [35] and Saeed *et al.* [36] reported resistance rates of 14.81% and 23.08%, respectively, in similar settings (Table 3).

In contrast, our study did not reveal any resistance, which may reflect the effectiveness of our treatment and patient-monitoring strategies. The absence of resistance observed in our study may also be explained by good control of treatment practices and rigorous patient follow-up, thereby reducing the risk of resistance development. This result underlines the importance of careful case management and adherence to anti-tuberculosis treatment, particularly in severe and complex forms such as pericardial tuberculosis.

The National Tuberculosis Control Programme (PNLAT) recommends the use of mycobacterial culture as the initial diagnostic test for pericardial samples in cases of suspected PTB [50]. Our results confirm this recommendation, with culture positivity observed in 75% of patients, highlighting its effectiveness.

It is important to note that these results are obtained only after several weeks of incubation, leading to delays in the management of this infection, particularly in cases of pericardial tuberculosis, which require early therapeutic intervention. This delay may have serious consequences for the patient's prognosis. In this context, the GX MTB/RIF test appears to be the method of choice, as it enables rapid and reliable diagnosis, thereby facilitating faster and more appropriate management of this form of extrapulmonary tuberculosis.

GeneXpert MTB/RIF remains an essential tool for the screening of tuberculosis (TB) and rifampicin-resistant tuberculosis (RR-TB), playing a key role on decisions regarding the initiation of treatment in cases of multidrug-resistant tuberculosis (MDR-TB) [51]. The World Health Organization recommended the use of the GeneXpert MTB/RIF test in 2010 for the diagnosis of pulmonary tuberculosis and in 2013 for the diagnosis of extrapulmonary tuberculosis [52].

Guidelines from the World Health Organization for the integration of the GeneXpert MTB/RIF test into the tuberculosis diagnostic process are based on its rapid results and proven performance, particularly its sensitivity and specificity for both pulmonary and extrapulmonary tuberculosis [53].

Limits of The Study

A major limitation of our study is the absence of confirmation of resistance cases detected by the GeneXpert test through a reference method such as GenoType MTBDR Plus. This limitation affects the accuracy of the results, particularly regarding the risks of false-positive and false-negative findings. In addition, the lack of data on patients' HIV status prevented assessment of the impact of TB-HIV co-infection on drug resistance.

Conclusion

The GeneXpert MTB/RIF test is superior to conventional methods (smear microscopy and culture) as an initial screening test for pericardial tuberculosis. Its good performance on smear- and culture-negative specimens demonstrates its effectiveness as a complementary diagnostic test. In the absence of limitations related to availability or cost, a single GeneXpert MTB/RIF test performed on a pericardial specimen could replace smear microscopy as the initial diagnostic method for PTB. Our results support the use of the Xpert MTB/RIF test as the first-line diagnostic tool for PTB, providing clinicians with a rapid and reliable method for the appropriate and rational management of anti-tuberculosis treatment.

Compliance with Ethical Principles

We declare that this study was conducted in accordance with the ethical principles of the Declaration of Helsinki and international ethical standards for biomedical research concerning human participants.

Participant Consents

We certify that all participants or their legal representatives provided informed consent prior to inclusion in the study.

Confidentiality and Data Protection

The personal information of the participants was anonymized and handled confidentially, in accordance with applicable data protection laws and regulations.

Conflicts of Interest

We declare that there are no conflicts of interest related to this study.

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Paucibacillary Cutaneous Tuberculosis Diagnosed Using GeneXpert MTB/RIF Ultra: A Case Report

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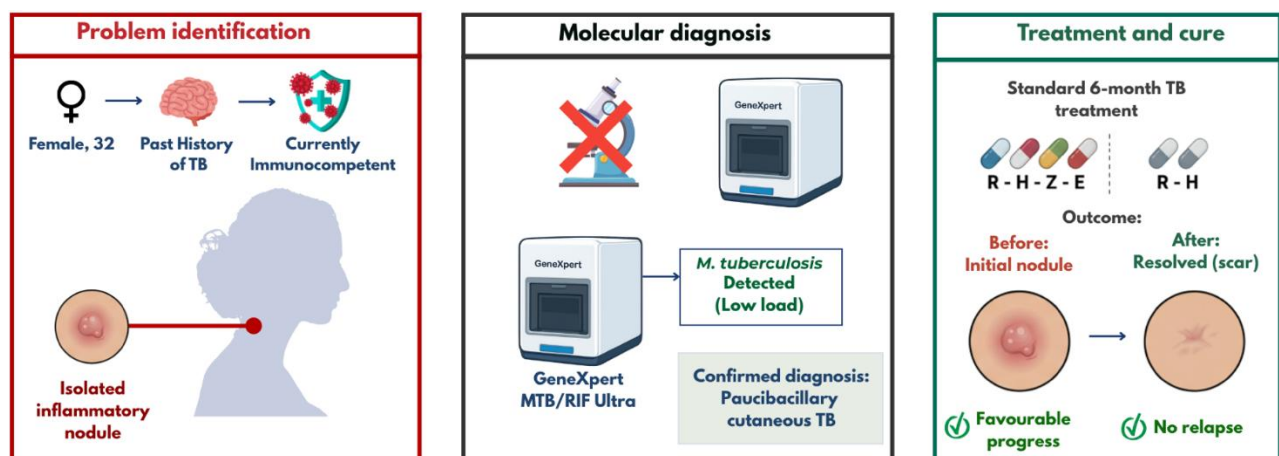
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Graphical abstract

Cutaneous tuberculosis is an uncommon paucibacillary form of extrapulmonary tuberculosis with diverse clinical presentations, complicating diagnosis. We report the case of a 32-year-old immunocompetent woman vaccinated with BCG and previously treated for neuromeningeal tuberculosis, who developed a progressive cervicothoracic skin lesion. Conventional microbiological tests were negative, whereas GeneXpert MTB/RIF Ultra on a skin biopsy detected *Mycobacterium tuberculosis* DNA without rifampicin resistance, confirming secondary scrofuloderma. Standard antituberculosis therapy led to a favourable outcome without recurrence.



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Abstract: Cutaneous tuberculosis is a rare form of extrapulmonary tuberculosis, often paucibacillary and with a polymorphic clinical presentation, making diagnosis difficult. We report the case of a 32-year-old immunocompetent woman, vaccinated with BCG, with a history of treated neuromeningeal tuberculosis, who developed a progressively worsening left cervicothoracic skin lesion. Clinical examination revealed an isolated inflammatory nodule without any alteration in her general condition. Conventional microbiological tests, including sputum smear microscopy and culture, were negative. But, molecular analysis by GeneXpert MTB/RIF Ultra on a skin biopsy detected *Mycobacterium tuberculosis* DNA at a low load, without rifampicin resistance, confirming paucibacillary cutaneous tuberculosis of the secondary scrofuloderma type. Standard antituberculosis treatment resulted in a favourable outcome without relapse. This case highlights the value of molecular techniques in the early diagnosis of atypical forms of extrapulmonary tuberculosis.

Keywords: GeneXpert MTB/RIF, Cutaneous Tuberculosis, Scrofuloderma.

Introduction

Tuberculosis (TB), a bacterial infection caused by the *Mycobacterium tuberculosis* (MTB) complex, remains a major public health problem worldwide, with a particularly high incidence in developing countries [1,2]. Pulmonary involvement is the most frequent form. Extrapulmonary manifestations, particularly those affecting the meninges, digestive system, osteoarticular system and urogenital system, present a diagnostic challenge because of their anatomico-clinical polymorphism [2,3].

Among these manifestations, cutaneous tuberculosis, most often caused by *Mycobacterium tuberculosis* but occasionally associated with *Mycobacterium bovis* [4], remains a rare entity, representing only 0.14% to 4.4% of tuberculosis cases. It can mimic numerous dermatological conditions, which may result in considerable diagnostic delay [3-5].

Despite the widespread introduction of BCG (*Bacillus Calmette-Guérin*) vaccination as a mandatory vaccine in the Moroccan National Immunization Programme (PNI), the protection conferred against extrapulmonary forms in adults remains variable and incomplete [6]. In addition, the occurrence of cutaneous forms in immunocompetent patients highlights the complexity of host-pathogen interactions [2,5].

The therapeutic protocol for cutaneous tuberculosis is based on prolonged antibiotic therapy combining several anti-tuberculosis drugs [1,2]. The diagnosis should therefore be considered in any chronic, destructive or verrucous skin lesion showing no signs of healing, even when conventional mycobacteriological tests remain negative [5,6].

We illustrate these particularities through the clinical observation of a young immunocompetent patient vaccinated with BCG, with a history of treated neuromeningeal tuberculosis, who subsequently developed paucibacillary scrofuloderma-type cutaneous tuberculosis. The diagnosis could only be confirmed through a molecular approach using the GeneXpert MTB/RIF Ultra test, despite negative bacilloscopy and culture results, reflecting the diagnostic difficulties and the importance of early management.

Clinical Observation

A 32-year-old woman originally from Morocco, vaccinated at birth with BCG as part of the National Immunization Programme, moved to Italy at the age of 21. At the age of 29, she was diagnosed and treated for neuromeningeal tuberculosis. She was initially hospitalized for

diffuse, intense and persistent headaches associated with a moderate but prolonged fever, followed by impaired consciousness progressing to coma.

The diagnosis was delayed because TB was not initially suspected in the non-endemic context of the patient's country of residence. Cerebral involvement was only confirmed after exploratory surgery, which identified an MTB infection. Following surgical intervention and the initiation of a well-managed antituberculosis treatment regimen, the patient's prognosis improved, leading to complete clinical recovery.

Twenty-two months later, the patient returned to the Kingdom of Morocco and subsequently developed a progressively worsening left cervicothoracic skin lesion. At the onset of the lesion, the patient, unaware of extrapulmonary forms of TB, began several forms of self-medication using ointments and creams obtained from a pharmacy without consultation or prescription. These included H1 antihistamines such as chlorphenoxamine, topical antibiotics such as fusidic acid and chlortetracycline, and topical antifungals such as clotrimazole and econazole. No therapeutic response or clinical improvement was observed, and the patient eventually consulted a dermatologist.



Figure 1. Clinical appearance of paucibacillary scrofuloderma-type cutaneous tuberculosis secondary to neuromeningeal tuberculosis in an immunocompetent patient vaccinated with BCG (2 weeks after onset).

Clinical examination showed that the patient was in excellent general condition, without fever or chills, but presented with a skin lesion in the form of an isolated nodule that had slowly developed over several weeks to more than one month. The lesion was located in the supraclavicular region opposite the left anterior cervicothoracic junction. It was a well-defined, rounded lesion measuring a few centimetres in diameter, slightly raised, with a smooth, shiny and reddish surface. The initial appearance suggested a benign inflammatory lesion resembling an insect bite, without crusting or scaling. The lesion was erythematous, reflecting a localized subcutaneous inflammatory process. It was slightly tender on palpation, without fistulization or discharge (Fig. 1).

In the context of a recent history of treated central nervous system tuberculosis (CNST), this clinical and anamnestic presentation, together with negative HIV serology, suggested secondary cutaneous TB despite the atypical clinical appearance of the lesion. The patient was referred for specialized bacteriological investigations. Initially, direct examination (bacilloscopy) and mycobacteriological cultures performed at a local medical biology laboratory on skin samples (swabs) were negative.

A mycobacteriological re-evaluation carried out at Ibn Sina Hospital, confirmed this paucibacillary profile, with absence of growth in culture on specific Löwenstein–Jensen medium and negative microscopy, showing no acid-fast bacilli (AFB) after modified Ziehl–Neelsen staining. These analyses were performed on a skin biopsy specimen of the lesion obtained by the treating dermatologist.

In contrast, molecular analysis using the GeneXpert MTB/RIF Ultra assay, performed on the same skin biopsy specimen, revealed within less than two hours the presence of *Mycobacterium tuberculosis* (MTB) DNA detected at a very low level, without detection of the *rpoB* gene associated with rifampicin resistance (RR), thus confirming the diagnosis of cutaneous tuberculosis. Additional investigations were recommended, but no other active systemic involvement was identified (Fig 2).



Figure 2. Molecular diagnosis performed on a skin biopsy specimen using the GeneXpert MTB/RIF Ultra assay, a real-time nested PCR technique enabling the detection of *Mycobacterium tuberculosis* and rifampicin resistance.

The diagnosis was scrofuloderma-type cutaneous TB. It was considered a secondary paucibacillary tuberculosis occurring in an immunocompetent individual vaccinated with BCG, in the context of post-treatment neuromeningeal tuberculosis. The patient was referred to a CDTMR (Centre for the Diagnosis and Treatment of Respiratory Diseases) in Casablanca to receive free treatment.

The therapeutic protocol consisted of standard four-drug antituberculosis therapy combining Rifampicin, Isoniazid, Pyrazinamide and Ethambutol for two months (2RHZE), followed by a four-month maintenance phase with Rifampicin and Isoniazid (4RH). The clinical course was favourable, with progressive regression of the skin lesion, although noticeable scarring remained (Fig. 3).

It should also be noted that the patient showed no recurrence of TB during follow-up, particularly beyond three months after completion of treatment. No cutaneous involvement or other extrapulmonary localization of tuberculosis was observed.

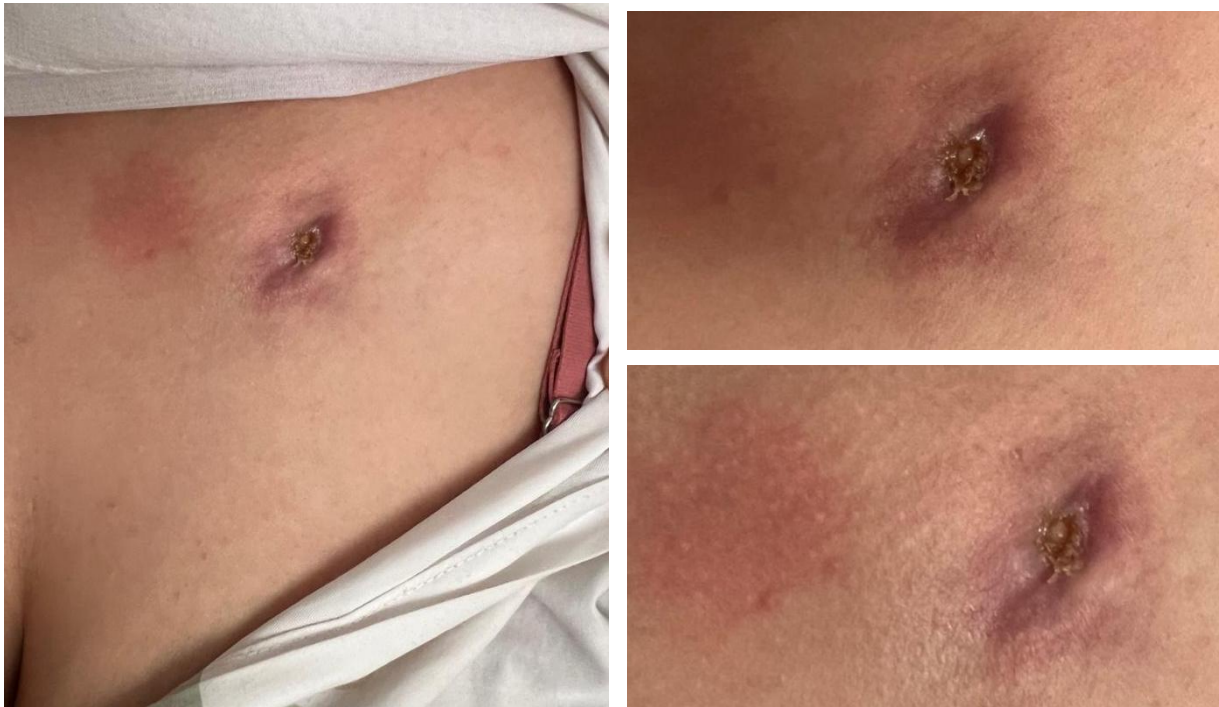


Figure 3. Clinical appearance of paucibacillary scrofuloderma-type cutaneous tuberculosis secondary to neuromeningeal tuberculosis in an immunocompetent patient vaccinated with BCG (after skin biopsy and at the initiation of antituberculosis quadritherapy).

Discussion

We report a clinical case of paucibacillary scrofuloderma-type cutaneous tuberculosis occurring in a young immunocompetent patient vaccinated with BCG at birth, in the particular context of a recent history of treated neuromeningeal tuberculosis, with diagnostic delay favoured by initial self-medication.

Cutaneous tuberculosis is a rare form of secondary tuberculosis, representing less than 1–1.5% of extrapulmonary TB cases [7]. Its polymorphic clinicopathological presentation depends on several factors, including the virulence of the *Mycobacterium tuberculosis* strain, the route of exogenous or endogenous contamination, and the immune status of the host [5,8].

Exogenous contamination mainly results from direct inoculation of *Mycobacterium tuberculosis* (Koch's bacillus) into the skin from the external environment, generally through a breach in the skin barrier such as a wound or trauma. This route of contamination occurs most often in non-immune individuals and may be responsible for clinical forms such as tuberculous chancre and verrucous tuberculosis, in which the skin lesion represents the primary site of infection [1,5,8,9].

In contrast, endogenous contamination results from dissemination of the tubercle bacillus from a pre-existing internal tuberculous focus, whether active or latent. This mycobacterial spread may occur through haematogenous, lymphatic or contiguous routes from a deep focus such as lymph node, osteoarticular or visceral tuberculosis, and is therefore responsible for secondary forms such as scrofuloderma or lupus vulgaris [5,8-10].

In our patient, the clinical history strongly suggests endogenous dissemination related to the previous neuromeningeal focus, raising the possibility of reactivation or secondary bacillary spread despite previously well-conducted treatment. This observation is consistent with data from the literature describing delayed secondary localizations after deep tuberculosis [5,8-10].

Scrofuloderma classically corresponds to the direct extension of an underlying tuberculous focus, which may be lymph node, osteoarticular or other deep involvement. Clinical forms without an identifiable active deep focus, as observed in our case, have nevertheless been described, suggesting either haematogenous dissemination or local reactivation of MTB. This hypothesis is reinforced by the 22-month disease-free interval following treatment for neuromeningeal tuberculosis [5,8-10] (Table 1).

Clinically, the location and misleading initial presentation of the skin lesion, mimicking a benign inflammatory condition, also contributed to delayed diagnosis. Early forms of scrofuloderma may present as non-specific nodules before progressing to the typical ulcerations and fistulas [5,8,9]. This variability in clinical presentation requires a high level of suspicion, particularly in patients with a history of tuberculosis. This explains the interest shown by the treating dermatologist, who suspected cutaneous tuberculosis and referred the patient for laboratory confirmation before initiation of the antituberculosis treatment protocol.

Table 1. Clinical forms of cutaneous tuberculosis [5]

Clinical form	Mode of acquisition	Main lesions
Lupus vulgaris	Primary or secondary infection by direct extension or lymphohaematogenous spread	Centrifugal extension, serpiginous plaques, purplish-yellow colouration, scaling, atrophy
Warty tuberculosis	Secondary infection by autoinoculation	Keratotic plaques with irregular borders and dystrophic scarring
Miliary tuberculosis	Primary or secondary infection by massive haematogenous dissemination	Diffuse papules followed by vesicles and crusts
Papulonecrotic tuberculid	Tuberculid occurring during the course of tuberculosis	Dermal papules followed by necrotic pustules and atrophic varioliform scars
Lichen scrofulosorum	Tuberculid associated with lymph node or bone tuberculosis	Lichenoid perifollicular papules and plaques, yellowish or reddish-brown
Tuberculous gumma	Secondary infection by haematogenous dissemination	Erythematous-violaceous subcutaneous nodules and ulcers
Bazin's erythema induratum	Tuberculid occurring during chronic tuberculosis	Bluish-red subcutaneous nodules, sometimes ulcerated, mainly on the lower limbs
Erythema nodosum	Tuberculid occurring during tuberculosis	Painful nodules
Tuberculous chancre	Primary infection by local inoculation	Painless ulceration
Scrofuloderma	Secondary infection by contiguous extension from a neighbouring focus	Erythematous-violaceous nodules, initially painless and mobile, later adherent with suppuration
Orificial tuberculosis	Secondary infection by autoinoculation from a primary focus	Nodules followed by painful ulcerations, abscesses and fistulae

In the Kingdom of Morocco, cutaneous tuberculosis remains relatively common, mainly represented by scrofuloderma and gumma tuberculosis, as demonstrated by hospital series from the university hospitals of Fez and Casablanca, with high proportions reaching 67% and 58% of cases, respectively [11]. This distribution contrasts with that reported in French and Chinese series, where tuberculids, particularly Bazin's erythema induratum, are more frequently observed [11]. These differences may be explained by epidemiological, immunological and environmental variations among the studied populations, as well as differences in bacillary load profiles and diagnostic contexts.

Although effective against severe forms of tuberculosis in children, particularly tuberculous meningitis, BCG vaccination does not prevent the occurrence of various extrapulmonary forms

in adults [7-9,12]. Several scientific studies have shown that BCG modulates clinical severity rather than the risk of infection, which could explain the paucibacillary nature and relatively localized course observed in our patient [7-9,12].

From a diagnostic standpoint, our clinical case illustrates the difficulty of mycobacteriological confirmation of paucibacillary cutaneous forms using conventional bacteriological diagnostic techniques. Negative smear microscopy and culture results on Löwenstein–Jensen medium are indeed frequent, with positivity rates below 30% in some series. According to the WHO, the sensitivity of culture on solid Löwenstein–Jensen medium is estimated at approximately 75–80% [5,8,13]. Similar findings have been reported in several recent studies, confirming that conventional microbiological diagnostic methods have limited yield in cutaneous tuberculosis, often making diagnosis difficult and delayed [8,14].

In this context, molecular techniques, particularly the GeneXpert MTB/RIF Ultra assay, play a crucial role. This real-time nested PCR enables rapid, sensitive and specific detection of mycobacterial DNA, even in samples with low bacterial load, with markedly higher sensitivity than microscopy and culture, considered the gold standard [1,13]. A meta-analysis by Denkinger *et al.* [15], comparing the technical performance of GeneXpert MTB/RIF with culture as the gold standard, confirmed its value in extrapulmonary forms, including cutaneous tuberculosis, with a significant improvement in diagnostic yield [15]. This comparative study reported a specificity of 98.7%, a sensitivity of 83.1%, and a high positive predictive value of 98% [15]. The presence of polymerase inhibitors in tested specimens, including biological fluids and biopsies, may explain the reduction in sensitivity observed in some situations [16]. In our case, the GeneXpert MTB/RIF assay nevertheless confirmed the diagnosis despite negative conventional tests and a low mycobacterial load in the specimen, findings that do not exclude the diagnosis of cutaneous tuberculosis.

Another important aspect of this clinical observation is the diagnostic delay associated with self-medication without medical consultation. Our patient applied several topical treatments, including antibiotics, antifungals and H1 antihistamines, without medical advice or a precise diagnosis. This contributed to masking the clinical signs and delaying specialized management. This phenomenon is widely described in developing countries, where unrestricted access to medications promotes diagnostic delay and encourages self-medication, with initial reliance on symptomatic treatments without medical consultation or prescription [17].

One study showed that the time to diagnosis (TD-TEP) for extrapulmonary tuberculosis is largely patient-related, with a mean of 31.23 ± 43.55 days and a median of 17.5 days. Several factors influence this delay, including advanced age, male sex, rural residence and lack of awareness of the disease [18].

Another Moroccan multicentre study conducted in 2021 revealed that the distribution of patients with cutaneous tuberculosis, according to the interval between lesion onset and the first medical consultation, was characterized by a mean delay of approximately 11.5 months, reflecting a particularly marked diagnostic delay. This prolonged delay is explained by several factors, including the initial neglect of lesions by patients because of their often painless and non-specific nature, together with limited awareness of this condition among primary care physicians. In addition, the waiting time for access to specialist consultation, which may range from 2 to 8 months depending on healthcare facilities and regions, further contributes to prolonged diagnostic delay [19].

From a therapeutic standpoint, management is based on the standard regimen recommended by the World Health Organization (2RHZE/4RH) and detailed in the National Tuberculosis Control Programme of the Moroccan Ministry of Health and Social Protection. This regimen remains effective in the majority of cases. The favourable outcome observed in our patient, with disappearance of the lesion during treatment, confirms the high sensitivity of the bacillus and the efficacy of the therapeutic protocol. Persistence of scarring nevertheless remains common, particularly in advanced cutaneous forms [20,21]. Finally, the absence of recurrence three months after completion of treatment, together with the negative staging investigations, confirms the isolated nature of this secondary cutaneous lesion. This observation underlines the importance of early diagnosis and appropriate therapeutic management in order to avoid complications, sequelae and recurrences.

On the occasion of World Tuberculosis Day on 24 March 2026, the Moroccan Ministry of Health and Social Protection issued a ministerial statement reminding the public that nearly 53% of tuberculosis cases in Morocco are extrapulmonary, emphasizing the importance of increased vigilance regarding these often misleading and atypical presentations [22]. In this context, it is essential that clinicians from all specialties systematically include extrapulmonary tuberculosis in the differential diagnosis of any chronic, atypical or unusual lesion, particularly as tuberculosis is endemic in the Kingdom of Morocco. Several studies have also shown that the concomitant association of pulmonary tuberculosis with a typical clinical presentation and an extrapulmonary form remains relatively uncommon [22].

Finally, this case highlights the major and indispensable contribution of molecular diagnostic tools, particularly the GeneXpert MTB/RIF assay recommended by the World Health Organization, which enables rapid, sensitive and reliable detection of *Mycobacterium tuberculosis*, including in paucibacillary forms, thus contributing to early and appropriate management [1].

Conclusion

This clinical case illustrates the diagnostic challenges associated with cutaneous forms of tuberculosis, particularly in their biologically paucibacillary and clinically atypical presentation in an immunocompetent individual vaccinated against tuberculosis. Our work highlights the point that negative results obtained with conventional bacteriological methods do not exclude the diagnosis and underlines the crucial contribution of molecular techniques, notably the GeneXpert MTB/RIF Ultra assay, for rapid and reliable confirmation.

The occurrence of secondary scrofuloderma after treated neuromeningeal tuberculosis also reminds clinicians of the possibility of late dissemination or reactivation, thus justifying prolonged clinical vigilance. In addition, the initial self-medication contributed to diagnostic delay, emphasizing the importance of greater patient awareness. Finally, this case underlines the need always to include extrapulmonary tuberculosis in the differential diagnosis of chronic skin lesions in endemic areas such as the Kingdom of Morocco.

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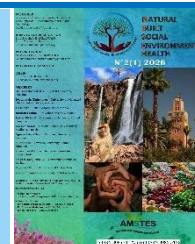
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Impact of storage temperature and duration on rRT-PCR Cycle Threshold (Ct) values in SARS-CoV-2-positive nasopharyngeal Samples: A study at a provincial hospital in Morocco

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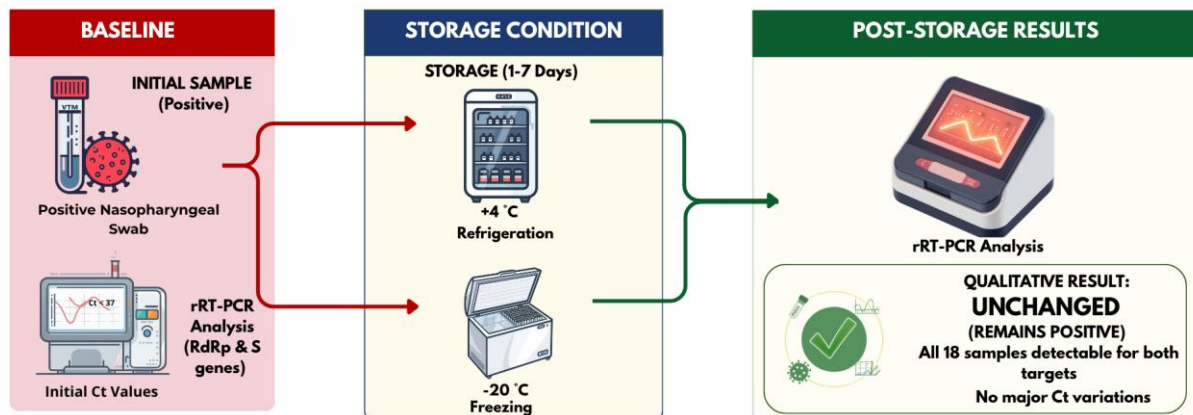
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Graphical Abstract

Accurate SARS-CoV-2 detection by RT-PCR depends not only on analytical performance but also on pre-analytical conditions. Sample collection, handling, and storage directly impact RNA integrity and cycle threshold (Ct) values, influencing diagnostic reliability.



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Abstract: Reverse transcription polymerase chain reaction (RT-PCR) is the gold standard for SARS-CoV-2 diagnosis of infection. Pre-analytical factors such as storage temperature and duration may affect RNA stability and, consequently, RT-PCR performance. This study aimed to assess the impact of these factors on cycle threshold (Ct) values to ensure reliable diagnostic results. Eighteen SARS-CoV-2-positive nasopharyngeal swab samples were stored at +4 °C and −20 °C and analyzed by RT-PCR over a period of 1 to 7 days. Detection of the S (Spike) and RdRp (RNA-dependent RNA polymerase) genes was performed, and Ct values were compared before and after storage. All analyses were conducted using the same standardized protocol for RNA extraction, amplification, and result interpretation. All initially positive samples remained positive after storage. Ct values showed only minor variations, with mean decreases of −1.02 cycles for RdRp and −1.05 cycles for the S gene after storage. Variations of up to 2–3 Ct cycles were observed in some samples. These findings suggest that short-term storage under standard conditions is unlikely to affect SARS-CoV-2 detection by RT-PCR. Because of the limited sample size, the analysis was mainly descriptive. Therefore, short-term storage of SARS-CoV-2 samples under routine laboratory conditions did not alter RT-PCR qualitative results.

Keywords: SARS-CoV-2, RT-PCR, CT value, Storage temperature, Storage duration.

Introduction

Since its emergence in Wuhan, China, in late 2019, SARS-CoV-2 rapidly evolved into a global pandemic that placed unprecedented demands on public health systems and diagnostic laboratories worldwide [1]. As the virus spread globally, timely and accurate detection became the hallmark of pandemic management, guiding clinical decision-making, contact tracing, and containment strategies [1]. Among the available diagnostic tools, real-time reverse transcription polymerase chain reaction (RT-PCR) has remained the gold standard for identifying active infections because of its high sensitivity in detecting viral nucleic acids [2].

The enormous number of clinical specimens, often reaching thousands per day in some regions, posed logistical challenges beyond laboratory testing capacity. Samples from areas without molecular testing capabilities are often transported to reference laboratories, where processing delays require controlled storage [3]. In routine diagnostic settings, laboratories frequently rely on temporary storage of samples prior to analysis, raising concerns about the reliability of RT-qPCR results under such conditions. These pre-analytical variables, including storage temperature and duration, are critical determinants of sample integrity and may influence the stability of viral RNA, potentially contributing to variability in RT-PCR results [4]. Despite the recognized importance of pre-analytical factors in molecular diagnostics, the specific impact of storage conditions on SARS-CoV-2 RNA stability remains insufficiently characterized. Most existing studies have focused on analytical performance or assay comparison. Only a limited number of studies have experimentally evaluated how storage temperature and duration influence viral RNA detectability under routine laboratory conditions.

In contrast, studies specifically addressing pre-analytical storage conditions have reported inconsistent findings. Some have suggested minimal RNA degradation under short-term storage. Others have indicated potential alterations in Ct values depending on temperature and duration. These discrepancies highlight the need for further investigation under real-world laboratory conditions, particularly in resource-constrained settings where immediate processing is not always feasible. Moreover, it is not clear whether short-term storage under commonly used conditions (such as refrigeration or freezing) significantly alters RT-PCR cycle threshold (Ct) values, which are often used as indirect indicators of viral load. In this context, describing Ct variations under different storage conditions may provide useful preliminary insights, even in the absence of inferential statistical testing. Addressing this uncertainty is essential to ensure the reliability and interpretability of molecular diagnostic results.

Based on these considerations, this study aimed to explore whether storage temperature and duration may influence SARS-CoV-2 RNA stability and result in variations in RT-PCR Ct values. This exploratory approach focuses on descriptive analysis of Ct value variations and qualitative RT-PCR outcomes under different storage conditions and durations.

This study investigates the influence of storage temperature and duration on the reliability of SARS-CoV-2 detection by RT-qPCR. Specifically, we evaluated whether these parameters affect viral RNA degradation and subsequent changes in Ct values, by analysing previously confirmed positive samples subjected to different storage conditions. This study provides descriptive observations intended to inform laboratory practices. By providing empirical data under real-world laboratory constraints, this study seeks to contribute to the optimization of sample handling protocols and to strengthen the robustness of molecular diagnostic practices. Understanding these effects is essential to ensure diagnostic reliability, maintain workflow efficiency, and support laboratory performance.

Materials and methods

This laboratory-based experimental study was conducted in the Microbiology laboratory of Hassan II Hospital, Settati, Morocco. Nasopharyngeal swabs were collected from COVID-19 contact cases in Settati between May 10 and June 10, 2021. During this period, 579 nasopharyngeal samples were collected, excluding duplicates, of which 72 tested positive for SARS-CoV-2 by RT-qPCR (Fig. 1).

All nasopharyngeal specimens included in this study were collected using standardized sampling kits consisting of a sterile nasopharyngeal swab placed in a tube containing viral transport medium (VTM). Contact cases were sampled at the Attanmya Center in Settati, a facility dedicated exclusively to COVID-19 screening, whereas hospitalized patients and individuals requiring emergency care were sampled directly at the hospital. Specimen collection was performed by personnel trained in nasopharyngeal sampling techniques and in accordance with recommended hygiene and biosafety measures. In all cases, sample packaging, transport, and storage procedures were strictly followed, including transportation to the laboratory in insulated containers to maintain appropriate conditions.

Samples were collected under routine diagnostic conditions, and initial Ct values showed variability between samples, reflecting differences in viral load among patients. After collection, nasopharyngeal swabs were transported to the laboratory in viral transport medium under refrigerated conditions (approximately +4 °C) and processed within a short time frame, in accordance with routine diagnostic procedures.

From each sample, 300 μ L of the transport medium was aliquoted into Eppendorf tubes and stored at +4 °C and –20 °C for variable durations. This approach was adopted to evaluate the effect of storage temperature and duration on SARS-CoV-2 RNA stability and RT-qPCR reliability under conditions where immediate processing is not feasible [5].

Aliquots were prepared to avoid repeated freeze–thaw cycles, and each aliquot was thawed only once prior to RT-qPCR analysis. Ct values were measured at baseline and after storage within the same specimen to assess the impact of storage conditions.

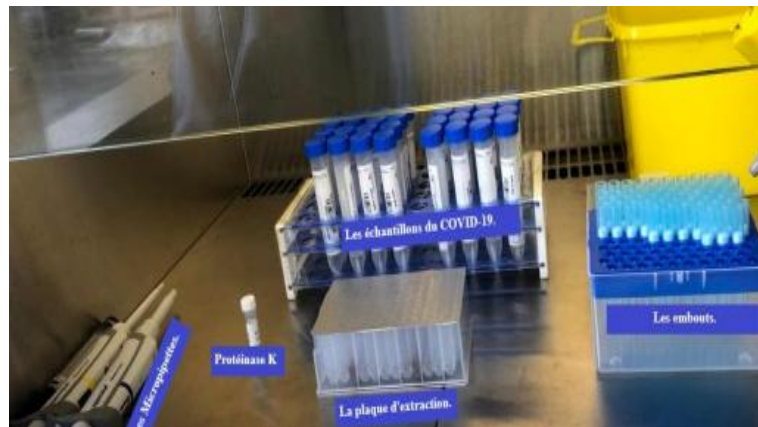


Figure 1. Pre-analytical setup for SARS-CoV-2 RNA extraction. Nasopharyngeal specimens, Proteinase K, extraction plate and pipette tips arranged for automated nucleic acid extraction prior to RT-PCR analysis.

Nucleic acids were extracted using the automated Big Fish® extraction system, based on magnetic bead technology. A volume of 200 μ L of each sample was processed according to the manufacturer's instructions. Proteinase K was used to enhance viral lysis. All procedures were performed under appropriate biosafety conditions to prevent contamination. The reaction mixture was prepared prior to amplification in a designated reagent preparation room. Master mix preparation was performed using the MAScIR SARS-CoV-2 M 2.0 kit. SARS-CoV-2 specific probes were labelled with the Cy5 fluorophore for the S gene, and the FAM fluorophore for RdRp gene.

RT-qPCR was performed using a qTower real-time thermal cycler system (Analytik Jena AG, Germany) (Fig. 2). A one-step real-time reverse transcription PCR assay was used to amplify simultaneously two SARS-CoV-2 target genes: the RNA-dependent RNA polymerase (RdRp) gene and the S (spike protein) gene, following the manufacturer's protocol (MAScIR SARS-CoV-2 Kit; OSANG Healthcare Co., Ltd., Korea). Results were interpreted according to the manufacturer's recommendations. SARS-CoV-2 positivity was confirmed by Ct values obtained for both target genes, with Ct values < 37 considered positive according to the kit guidelines.



Figure 2. Real-time RT-PCR workstation used for quantitative detection of SARS-CoV-2 RNA and monitoring of cycle threshold (Ct) values for the RdRp and S gene targets under different storage conditions.

Positive and negative controls were included in each run to ensure assay validity. An Internal Control (IC) was employed to monitor the efficiency of RNA extraction and the amplification process (Fig. 3). The IC is expected to yield a positive result in all valid patient samples, confirming proper extraction, absence of significant PCR inhibition, and correct execution of the analytical procedure.

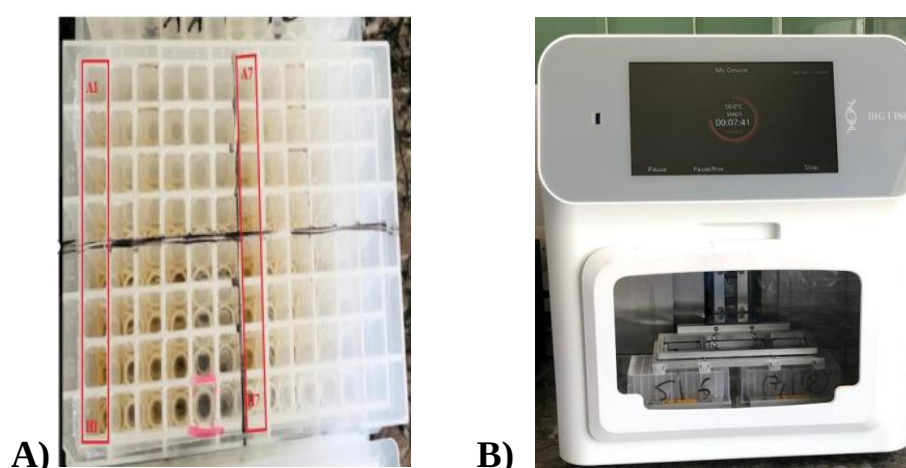


Figure 3. RNA extraction workflow for SARS-CoV-2 detection using the Big Fish® automated extraction system. **(A)** Extraction plate containing nasopharyngeal specimens and reagents arranged for automated magnetic bead-based RNA extraction. **(B)** Big Fish® automated nucleic acid extraction system used for the purification of SARS-CoV-2 RNA prior to RT-qPCR analysis.

Statistical analysis

Data were analysed using IBM SPSS software (version 27). Given the small sample size ($n = 18$) and the repeated-measures nature of the data, a mainly descriptive approach was adopted.

Ct values before and after storage were summarized using mean $\pm$ standard deviation (SD) and range. Variations in Ct values were assessed descriptively across storage temperatures ($+4\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$) and durations (1 to 7 days).

The analysis focused on evaluating the stability of RT-qPCR results, particularly the preservation of Ct values below the positivity threshold ($\text{Ct} < 37$). No inferential statistical tests were performed because of the limited sample size, in order to avoid overinterpretation of the results.

Results

In the present study, 18 nasopharyngeal swab samples were analysed to evaluate the effect of storage temperature and duration under real-world pre-analytical conditions, which represent a major source of variability in molecular diagnostics.

RT-PCR analysis demonstrated that all initially positive samples remained positive despite variations in storage conditions. Table 1 presents the cycle threshold (Ct) values according to storage duration (1 to 7 days) and temperature conditions ($+4\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$). All SARS-CoV-2-positive samples remained positive ($\text{Ct} < 37$), with Ct values remaining within a comparable range across storage conditions, indicating overall stability of viral RNA. But, variations of up to 2–3 Ct cycles were observed in some samples, depending on storage conditions and duration. These variations, although not affecting qualitative interpretation, may be relevant in molecular diagnostics, as Ct shifts of this magnitude can reflect potential changes in viral RNA quantity or sample integrity. This variability highlights the importance of considering pre-analytical conditions when interpreting Ct values, particularly in clinical or borderline cases. Both target genes remained detectable when initially present. In some cases, only one target gene was detected. For example, in sample no. 8040, the S gene was not amplified; and this result remained unchanged after 24 hours of storage.

Ct values showed minimal variation after storage. Mean Ct values slightly decreased from 26.85 to 25.83 ($\Delta\text{Ct} = -1.02$) for RdRp and from 25.20 to 24.15 ($\Delta\text{Ct} = -1.05$) for the S gene. All Ct values remained below the positivity threshold ($\text{Ct} < 37$), with no qualitative change, observed

after storage. The results are summarized in Table 1, which includes laboratory identification codes assigned to each sample to ensure data traceability.

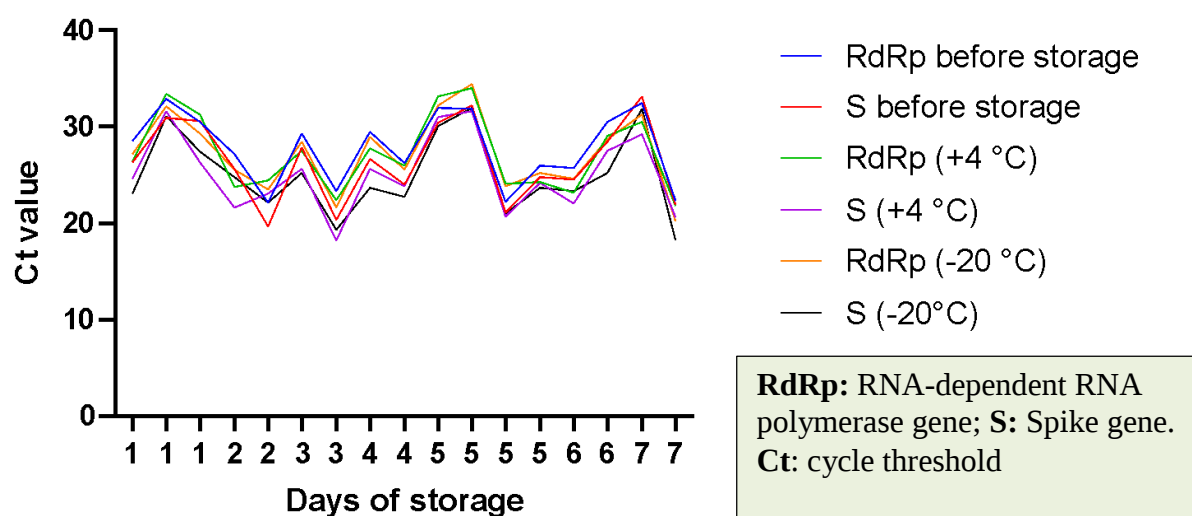


Figure 4. Comparison of RdRp and S gene Ct values before storage and after storage at +4 °C or −20 °C for 1–7 days in SARS-CoV-2-positive nasopharyngeal samples.

The graph illustrates the Ct values of the RdRp and S genes measured in SARS-CoV-2-positive nasopharyngeal samples before storage and after storage under two temperature conditions (+4°C and −20°C) over a period ranging from 1 to 7 days. Overall, the Ct values for both genes remained relatively stable across all samples and storage conditions, with no marked shifts observed between pre-storage and post-storage measurements.

Similarly, no consistent differences were detected between samples stored at +4°C and those stored at −20°C, suggesting that both storage conditions preserved the integrity of viral RNA within the studied time frame. The patterns observed for RdRp and S genes were comparable, indicating that gene detection was not differentially affected by storage temperature or duration.

These findings suggest that, under the conditions tested, short-term storage of nasopharyngeal samples at either +4°C or −20°C does not substantially impact Ct values for RdRp and S targets in SARS-CoV-2-positive specimens.

Discussion

Reverse transcription polymerase chain reaction (RT-PCR) remains the gold standard for the diagnosis of SARS-CoV-2 infection due to its high analytical sensitivity and specificity [6]. During amplification, the cycle threshold (Ct) value, which is inversely proportional to viral load,

is commonly used as a surrogate marker of viral RNA quantity. But, Ct values may be influenced by both analytical and pre-analytical factors. Among these, pre-analytical variability including sample collection, handling, storage temperature, and storage duration, represents one of the most important sources of variability in currently used molecular diagnostic procedures [7, 8].

In this study, we evaluated the effect of storage temperature and duration on the stability of SARS-CoV-2 RNA in nasopharyngeal swab samples collected at the medical analysis laboratory of Hassan II Hospital, Settat. Our data clearly indicate that SARS-CoV-2 positive samples remained positive after up to seven days of storage, regardless of the storage temperature (+4 °C and -20 °C). Importantly, no major changes in Ct values were observed for either the RdRp or S target genes.

These observations are consistent with previously published studies demonstrating that SARS-CoV-2 RNA remains stable under various storage conditions. In particular, minimal variations in Ct values have been reported for nasopharyngeal swab samples stored at +4°C for a few days, particularly if such samples were conserved using suitable transport or extraction buffers [9, 10]. In line with our results, previous studies have reported that SARS-CoV-2 RNA remains stable under both refrigerated and frozen storage conditions, supporting the robustness of RT-PCR detection across different laboratory contexts [5,11]. Despite the overall stability observed, variations in Ct values of up to 2–3 cycles were reported under certain pre-analytical conditions. These changes are consistent with a reduction in detectable viral RNA, probably related to sample handling and storage conditions affecting RNA integrity [12]. Although these variations did not affect the qualitative interpretation of results in our study, they may be clinically relevant in specific situations, particularly for samples with low viral loads near the detection threshold. This finding underscores the importance of cautious interpretation of Ct values in clinical practice. In the context of patient follow-up, adjustment of the therapeutic regimen and other medical decisions should not be based solely on Ct values, as these may be influenced by multiple pre-analytical and analytical factors.

The pre-analytical phase, particularly sample collection, is highly important in the accuracy of RT-PCR assays. Proper nasopharyngeal sampling is essential to ensure sufficient human cellular material, which is indirectly verified through internal control amplification. Insufficient amplification of the human target gene is considered an invalid result and is more likely attributable to poor sample collection rather than a low viral load.

Previous studies have highlighted that pre-analytical factors can influence the reliability of SARS-CoV-2 RT-PCR testing and should therefore be carefully controlled throughout the

diagnostic process [13]. In addition to sample collection, RNA transport and storage conditions play a crucial role in preserving RNA integrity. Delays in sample processing, temperature fluctuations, and repeated freeze–thaw cycles may potentially lead to RNA degradation. However, the results of the present study indicate that when samples are transported under standard conditions and stored appropriately, viral RNA integrity is well preserved, as reflected by minimal variations in Ct values. These findings are consistent with previous studies, which reported no apparent loss of detection sensitivity after short-term storage at 4 °C and –20 °C [14].

Interestingly, samples showing detection of a single viral gene prior to storage maintained the same detection pattern after storage. This observation suggests that storage conditions do not selectively affect specific genetic targets, but rather preserve the molecular profile of the sample. This is particularly relevant for samples with low viral loads, where even minor degradation could potentially affect detectability. Although storage conditions did not significantly influence Ct values in this study, other factors may contribute to variability. These include the presence of PCR inhibitors such as mucus, blood, or certain medications, which may interfere with amplification efficiency and lead to increased Ct values or amplification failure [15]. Nevertheless, this study has several limitations that should be acknowledged. First, the sample size was relatively small ($n = 18$), which may limit the generalizability of the findings. This is mainly caused by the fact that limited residual volumes were available following routine diagnostic procedures. Additionally, a number of samples were referred to the National Institute of Hygiene (INH), Morocco, for sequencing, which further reduced the pool of specimens accessible for this study. Furthermore, some samples exhibited high viscosity, complicating accurate aliquoting and consistent handling, and they were therefore excluded from analysis.

Second, no inferential statistical analyses were performed because of the limited sample size, which restricts the ability to assess statistical differences. Third, the observed Ct variability, although not affecting qualitative results, may still be relevant in borderline cases. Finally, this study did not assess the impact of repeated freeze–thaw cycles or long-term storage beyond seven days, which could further influence RNA stability.

Therefore, the conclusions of this study should be interpreted with caution. Although our findings suggest that short-term storage under standard conditions does not compromise SARS-CoV-2 detection, we cannot exclude the possibility of quantitative variability under different or more extreme conditions.

Overall, the results of this study confirm that, when pre-analytical parameters are adequately controlled, variations in storage duration and temperature do not appear to have any substantial

effect on the qualitative outcome of SARS-CoV-2 RT-PCR testing. These findings suggest a degree of operational flexibility in sample storage under routine diagnostic conditions, particularly in high-throughput or resource-limited settings. They also highlight the importance of careful interpretation of Ct values and strict control of pre-analytical parameters, including sample quality and the presence of potential PCR inhibitors, to ensure optimal test sensitivity and reliability.

These constraints may have reduced the statistical power of the study and may limit the generalizability of the findings. As such, the results should be interpreted with caution, and further investigations of larger samples are recommended to validate these findings.

Conclusion

All initially positive samples remained positive throughout the study period, with only minor variations in Ct values for the RdRp and S target genes under both storage conditions. These findings suggest that short-term storage temperature and duration are unlikely to affect SARS-CoV-2 RT-PCR detection under routine laboratory conditions. Further studies with larger sample quantities (to allow for high, low and borderline Ct values) are warranted to confirm these findings.

Acknowledgments: We thank all the staff of the Microbiology laboratory of Hassan II Provincial Hospital, Settat, for their valuable support and assistance.

Ethical Approvals: This study did not include patient data or identifiable personal information. Only anonymized SARS-CoV-2-positive nasopharyngeal samples were used for technical analysis. Administrative authorization was obtained from Hassan II Provincial Hospital, Settat, prior to conducting the study.

Code availability: Not applicable

Conflict of interest: The authors declare that they have no conflict of interest

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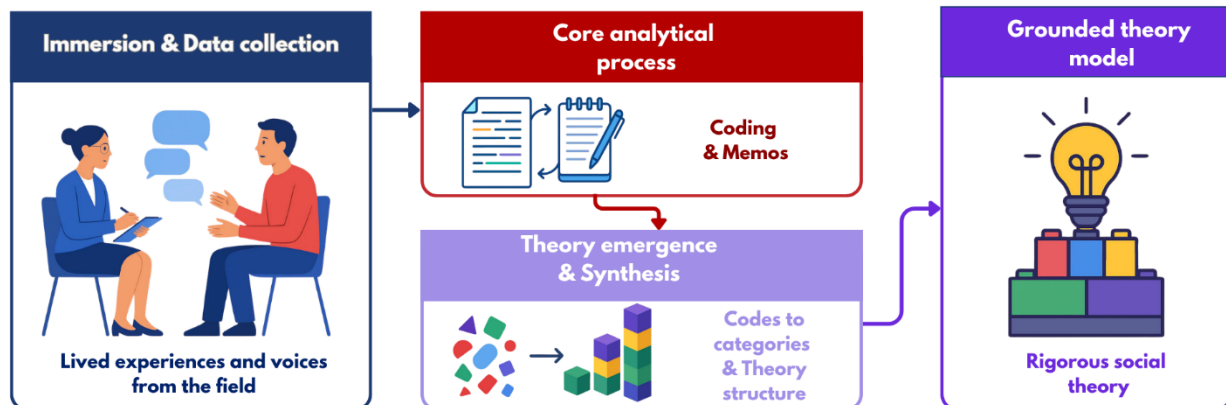


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BOOK REVIEW



Grounded Theory in Action: Voices from the Field, edited by Gregory Hadley and Antony Bryant, **authors:** Babchuk, W. A., Lynch-O'Brien, L. I., Friese, S., Unterpertinger, E., Thornberg, R., Horton, P., Forsberg, C., Engward, H., van Veggel, N., Allison, J., Mahmood Zada, A., Urquhart, C., Miller De Rutté, A. M., Bachynski, J. C., Costa, I. G., Long, A., Keane, E., Grant, M. J., Gibson, B., Morgner, C., Redway, A. M., Kemm, R., Cooper, K. Cheltenham: Edward Elgar Publishing, UK, 2026, 192 pp., £85.50 (hardback), ISBN: 978-1-03532-190-2.

The pioneers of artificial intelligence, such as Alan Turing and John McCarthy in 1956, began questioning the possibility of designing machines “capable of thinking”. Today, since the revolutionary launch of ChatGPT by OpenAI on 30 November 2022, we face an uncontrollable technological force, appearing under different names, through several inventors and several major powers, made available to the general public, for both the very young and the very old alike, across all categories. Several already see in it a form of refuge in the face of human failing. Others fear a progressive erosion of human experience, of human knowing-how-to-be and knowing-how-to-do, deepening this “desert of ourselves”.

And as the current algorithmic era seems to weaken certain human dimensions across many fields, we now need the means to move forward with greater security, through research strategies centred on human experience and social dynamics, in order to ensure a form of

rebalancing. This book makes a worthy contribution. This research proposes a strategy of listening, understanding lived human experience and reconstructing meaning. Grounded Theory thus appears as a form of remedy against faults in this algorithmic era, founded upon automation, speed, prediction and standardization.

I have often considered that these types of published academic research escaped classical methodological frameworks, and this has led me to focus on their deeper nature. I refer to works that emerge from reality, are rooted in lived experiences, and seek less to measure than to understand. I am thinking particularly of those qualitative studies exploring the psycho-social dimensions of quality of life among patients affected by breast cancer, or those concerned with the psychological impact of caesarean delivery, describing emotions, suffering, reactions to diagnosis, and family and social dynamics. Others investigate the effects of heavy treatments, such as chemotherapy or radiotherapy, on everyday life, or give voice to more silent experiences, including trauma linked to sexual abuse among adolescent girls and the need for therapeutic support to sustain their mental health and well-being.

Such research is indispensable. It addresses highly sensitive human dimensions within the system, draws upon diverse social phenomena in order to construct theories that are both useful and applicable, deserves particularly careful attention, and requires, for its implementation, an adaptive approach, constructed by the researcher in close connection with the context, the field, and dynamic data capable of evolving throughout the course of the research itself. *Grounded Theory in Action* fills this gap by proposing a genuine structuring of these approaches, which are often perceived as intuitive.

Grounded Theory was developed by Barney Glaser and Anselm Strauss in 1967 (p. 37) [1], and this approach provides a methodological response that also makes it possible to explore mechanisms of adaptation, strategies of resilience, and the social interactions constructed around complex situations, allowing explanatory models to emerge directly in relation to their contexts and specific challenges. The originality of this book lies in the way it renders the approach accessible, intelligible, and above all applicable. The work emphasizes that this emergent approach, far from being informal, rests upon a rigorous and reproducible logic capable of transforming data derived from reality into a solid and scientifically defensible theory. Even today, this theory remains insufficiently mobilized as a structuring methodological

framework, or is applied without fully mastering its principles, despite the fact that these principles remain essential for the production of rigorous and fruitful results.

I have often attempted to compare these types of research with my own practices. I frequently mobilize multidimensional data analysis tools, as well as data mining tools, which make it possible to “fish out” hidden information from a gigantic data matrix. This approach relies upon algorithmic and quantitative logic, where patterns emerge from calculations and correlations, and it is something entirely different. But, the logic of Grounded Theory recalls that several major scientific ideas were born from careful attention paid to reality and observed phenomena, as illustrated by the famous intuition associated with Isaac Newton observing the fall of an apple [2], or that of Joseph M. Juran, who, by connecting himself to everyday events and the industrial world, developed the idea of applying the principle introduced by the Italian economist Vilfredo Pareto and his 20/80 rule [3].

The quantitative and qualitative approaches sometimes appear to overlap, and both seem to be triggered through a connection with the field, its “Voices”, and the researcher’s “intuition”. In the genesis of quantitative research, even before the formalization of hypotheses and protocols, the researcher often passes through a phase of observation, connection with the field, and perception of emerging issues. Reality therefore appears to precede measurement, as though certain quantitative approaches found, at their origin, an initial anchorage in experience and environment.

Grounded Theory nevertheless remains a highly distinct method, transforming lived experiences and field data into a structured theory through its precise tools of analysis and interpretation (pp. 69–79). After the phase of immersion and observation in the field (pp. 37–45) come data collection through sampling, interviews (pp. 48–59), line-by-line analysis of statements, comparison of responses, the development of analytical notes, and eventually the hierarchical organization of responses into categories (pp. 103–113). The theory then begins to emerge.

Other associated tools are integrated, particularly coding (pp. 92–102), which makes it possible to extract the invisible fibres contained within discourses, testimonies, and the whole body of observations. Each of these elements must be assigned labels. When a patient declares, “I no longer recognize myself after the operation”, this may be coded as a loss of identity, or an

alteration of body image. Coding therefore fragments meaning and facilitates understanding. Susanne Frieze and Erika Unterpertinger (pp. 23–33) warn us against an excessive dependence on CAQDAS tools in qualitative research, at the risk that the machine may begin to think in place of human beings and orient their decisions.

The researcher then enters a more reflexive phase, mobilizing other tools in order to understand the phenomenon better. Several interviewed cases may describe the same feeling in different and distinct ways, which then becomes necessary to clarify. Other analytical mechanisms subsequently frame and reinforce this specificity. Alongside “coding”, “memos” also intervene, stimulating the birth of theory. Memos do not constitute free opinions, but rather analytical reflection tools linked to the data and the field, even if each author possesses a writing style of his or her own. They consist in refining reflections, establishing links between concepts, as well as between causes and effects (p. 116). Once the first significant elements of the studied phenomenon have been identified, the approach may call upon theoretical sampling, targeting the strata or specimens likely to provide an even more precise and specific understanding of the phenomenon under study.

During the official launch of the French strategy on artificial intelligence, Emmanuel Macron presented, during the event entitled *AI for Humanity*, organised on 29 March 2018 at the Collège de France, following the publication of the report by Cédric Villani, four major strategic pillars [4]:

- a.) strongly support scientific research;
- b.) develop laboratories, universities and innovation centres;
- c.) attract high-level researchers, engineers, students and experts;
- d.) make France attractive to international talent in the field of AI / IA.

The practice of Grounded Theory particularly supports scientifically framed research on ethical, political and societal levels, in direct resonance with the first and fourth pillars of the strategy presented during *AI for Humanity*.

At a time when AI is silently damaging essential human functions, standardizing human knowledge and behaviour, “Giving meaning to artificial intelligence” [5] also means reconnecting our research to lived experiences, making it attentive to reality, respectful of human experiences and firmly oriented towards understanding. Thus, the human and qualitative

sciences seem naturally to be becoming more necessary than ever. *Grounded Theory in Action* may frame approaches that seek to reintroduce essential human dimensions.

Grounded Theory in Action reminds us that understanding the human being, listening to lived experiences and reconstructing meaning from reality perhaps remain among the most essential scientific functions to preserve.

This practical reference work, overflowing with concrete examples and fascinating case studies, directs our attention towards the way in which we produce and value knowledge, as a scientific posture in its own right.

The book also offers elements for a useful checklist for good editorial practices. This book encourages us to adjust the author guidelines and to integrate Grounded Theory (GT) as a methodological reference, because it can deeply structure scientific, editorial, and ethical quality.

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**Natural Built Social Environment Health**

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www.nbseh.org**BOOK REVIEW**

Advances in Temperate Agroforestry, Edited by Maria Rosa Mosquera-Losada, Universidade de Santiago de Compostela, Spain; Ladislau Martin, Embrapa, Brazil; Anastasia Pantera, Agricultural University of Athens, Greece; Allison Chatrchyan, Cornell University, USA. ISBN: 9781801467193, £145.00, 2025, 354 pp. (Series No. 156).

This book completely surveys temperate agroforestry systems. These now receive more attention as we face combined challenges from climate change, soil degradation and food insecurity. The book reflects the multi-dimensional nature of agroforestry research and brings together contributions from different fields (including agronomy, ecology, forestry, soils or environmental economics) into a single volume.

This book identifies a key problem: Traditional agricultural systems based on specialization and intensification are approaching their limits of sustainability. The shrinking of biodiversity, soil organic matter loss and increased vulnerability to climatic stress all signal a call for wider integration. In this sense, temperate agroforestry is not merely a small-scale sub-strategy but an innovative intermediate system that combines aspects of agricultural production with the provision of functions and services provided by trees in natural ecosystems.

One of the book's strengths is its multi-scale perspective. Instead of being restricted to field-level processes, the authors generalize up to landscape level issues pertaining to ecological connectivity and ecosystem service provision. Seen in this broader context, agroforestry works well as a way of organizing agricultural landscapes; it is more than merely practices and approaches that can be applied at a local scale.

The chapters reviewing silvoarable systems, with a particular focus on alley cropping, give in-depth information about tree–crop–soil interactions. They document positive impacts on soil structure, water infiltration and erosion control. Other microclimate regulation by trees includes reduced evapotranspiration and protection of crops from wind. The authors do not ignore the complexity of these systems and the fundamental difficulty of competition for light and water.

Likewise, the discussions on silvopastoral systems reflect both opportunities and limitations/constraints. Planting shade trees in grazing systems can have benefits for animals (reducing heat stress) and land productivity. But mechanization, and the trade-off between forage produced against tree growth have been identified correctly as possible constraints.

The book also makes an important contribution to the topic of ecosystem services. Carbon sequestration, biodiversity and soil fertility are the subjects of several chapters that demonstrate how agroforestry systems can play a significant role in climate change mitigation. The results seem extremely context-dependent, varying with climate, soil types and species choice. Broad-scale generalizations are difficult.

The modelling and simulation tools are methodologically important because they allow us to explore long-term system performance under all kinds of management strategies. But, these models typically depend on oversimplified approximations of reality and may not be able to describe the complexity of biological interactions present in real systems.

The economic part is introduced progressively. Several chapters evaluate the economic viability of agroforestry systems and indicate that, although they may entail steep start-up costs with delayed payoffs, they could provide more stable long-term incomes. These analyses do not all include transition costs or the institutional barriers for farmers and so are of limited practical relevance.

Since this book is largely centred on Europe and northern North America, it is uncertain to what extent its conclusions will apply elsewhere, in regions such as Mediterranean, tropical or dryland environments that can impose quite different constraints [1]. Furthermore, the social or anthropological aspects are fairly under-researched too. Data on such matters would enrich these analyses. Farmer perceptions, adoption dynamics and land governance are easily glossed over but are crucial for understanding why agroforestry is rarely adopted in practice. Perhaps these matters can be included in a revised edition of this book, which could perhaps also be supplied with a more ‘applied’ character, to commend the data to practitioners and policy-makers.

In general, researchers will find this to be a major work. Not only does it compile existing knowledge but it also identifies key priorities for the future research agenda in relation to optimizing agricultural systems under environmental change. Thus, the book makes a very strong case for Agroforestry as playing an integral role in sustainable agricultural systems rather

than just being a small player. Nevertheless, it would improve its global relevance if the focus were widened to include more socio-economic contexts and perspectives.

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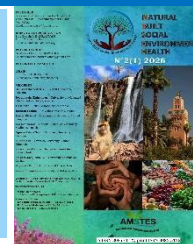
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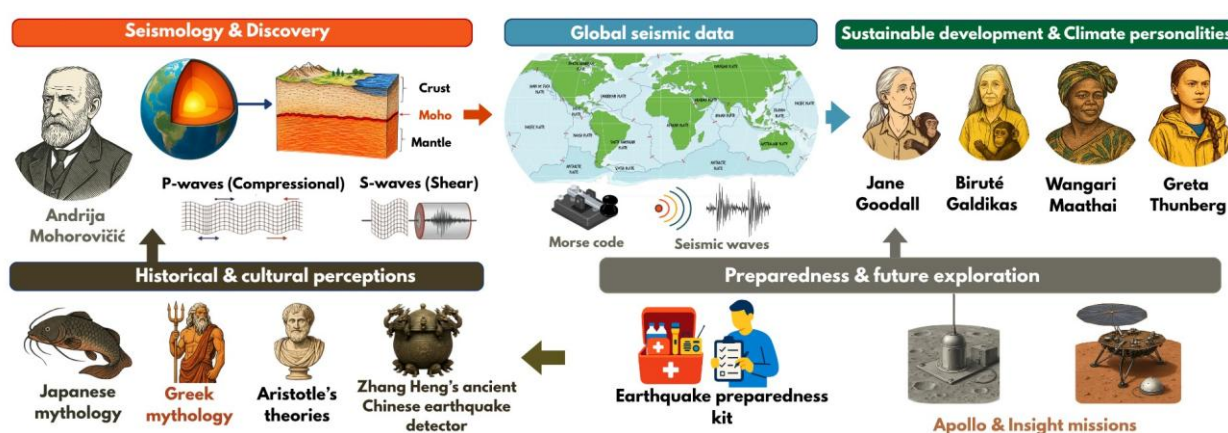
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BOOK REVIEW

When Worlds Quake: The Quest to Understand the Interior of Earth and Beyond, by Hrvoje Tkalčić. Princeton: Princeton University Press, 2026, 312 pp., \$29.95/£25.00 (hardcover), ISBN: 9780691271477.



Every exceptional person often carries within his or her heart a secret source of inspiration that nourishes the creativity and shapes the originality of the works. For a painter, this may be an imaginary landscape glimpsed somewhere between “perception and memory, something like Monet’s *Impression Sunrise* [1]. For a poet or a romantic singer, this may be a certain “Madeleine”, that silent figure of Absence and Waiting, the beloved woman who never comes, sometimes more alive in the heart than in reality [2]. For an inventor, this may be a luminous idea patiently pursued throughout life, like a distant star, much like Nikola Tesla pursuing an almost utopian vision of universal energy [3]. And for Hrvoje Tkalčić, one senses in this book that he feels a particularly deep admiration for Andrija Mohorovičić (Moho), who seems silently to accompany the entire narrative (pp. 37–64).

Moho (1857–1936) was a Croatian scientist, like Tkalčić, fascinated by the vibrations of the Earth. He was behind the discovery of the discontinuity between the Earth’s crust (the outer layer where we live) and the deep mantle (denser and hotter), enabling humanity better to understand how and why seismic waves suddenly changed speed at a certain depth, and this

indeed occurs during the crossing of this boundary. This layer still bears his name to this day: “the Moho” (Figure 1).

Andrija Mohorovičić represents, for the author, and for the future, the symbol of an intellectual curiosity capable of crossing the boundaries of monodisciplinarity. Hrvoje Tkalčić does not speak of Andrija Mohorovičić as a simple scientific name in this work. He speaks of him with affection, respect and intellectual admiration, and this can be felt throughout several passages. It is only natural, since Mohorovičić, using only simple means that were still poorly developed in his time, succeeded in bringing forth a theory that would profoundly transform our understanding of the Earth. The author of the book indeed resembles him greatly, and this is probably why Tkalčić allows this feeling of particular closeness towards him to emerge. And throughout the book, just like him, he constantly insists on the importance of open and interdisciplinary thinking, and of scientific imagination, in stimulating creativity.

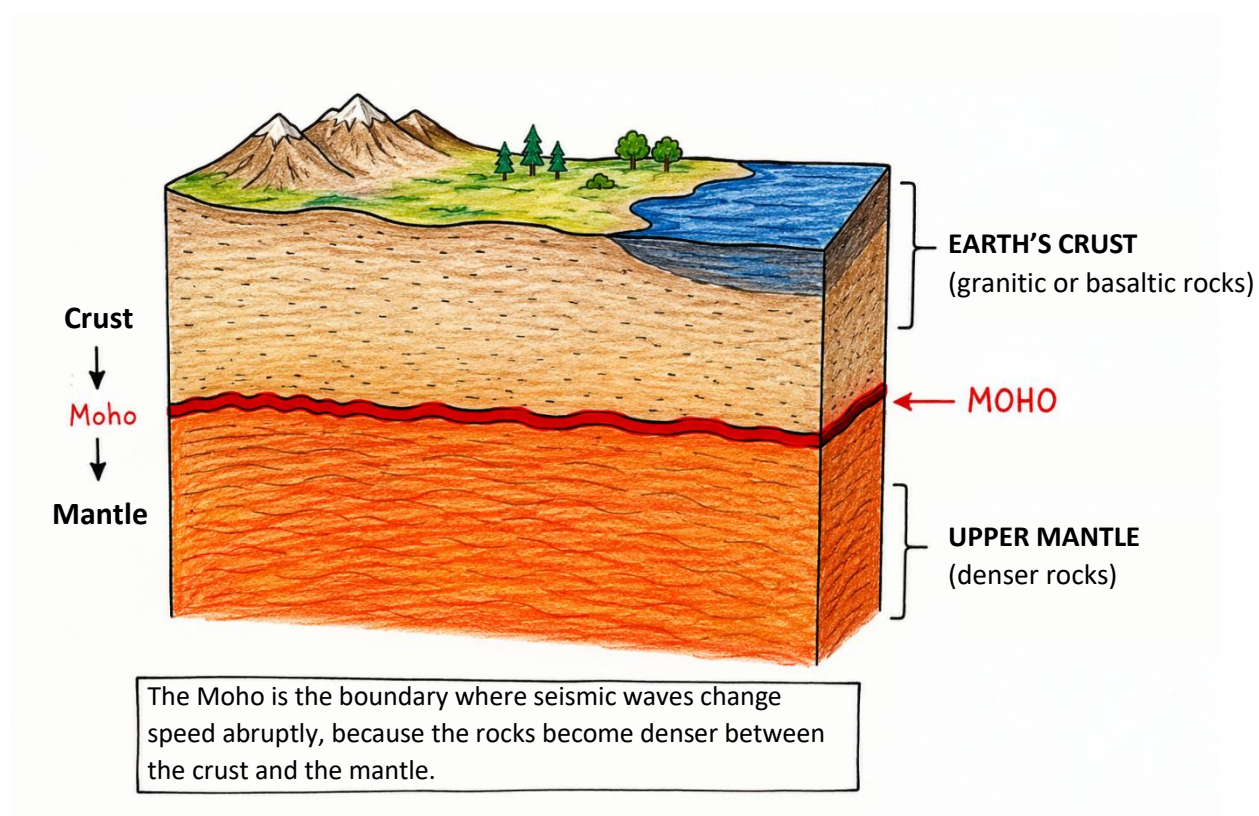


Figure 1. The Mohorovičić discontinuity (Moho), marking the boundary where seismic waves abruptly change velocity owing to the increase in rock density between the Earth’s crust and the mantle

This book vibrates with joy, to the rhythm of the “P” and “S” waves of an earthquake (pp. 37–64); each pulsation, each impulse seems to express itself in Morse code in order to allow the

“Sharp Look Inside” (p. 118) to discover gradually the secrets hidden beneath our feet. With each completed chapter, something changes in our perception: the ground beneath our feet appears to be moving... The mountains seem slowly to breathe... The oceans become witnesses to a very ancient past... And the stars remind us that the Earth began long before us, somewhere within the luminous dust of the universe (pp. 12–22), reminding us... reminding us... of all the other silent stories.

In order to manage pain or fear, one must mentally enter into the very mechanism that causes them: imagine the movement, the sound, the gesture, and almost physically understand the action taking place. This is how I manage, for example, to tolerate an injection, or even stage fright during a plenary address; instead of fleeing from the sensation, I enter into it with the mind, and the pain then becomes far more bearable.

Tkalčić never seeks to frighten when he evokes or predicts terrible earthquakes that caused major damage in the past, such as the 1906 San Francisco earthquake (pp. 23–36), sometimes accompanied by tsunamis (pp. 158–178). On the contrary, Tkalčić chooses very quickly to reveal the associated mechanism, as if to help master emotions before progressively enlightening us. He writes, for example, that “earthquakes simply come from blocked energy”. Or again, that natural disasters have not only destroyed the Earth; they have also contributed to the gradual construction of a habitable planet and still continue today to maintain its balance.

The book also explains triangulation in a very elegant manner, showing how, with a little time and mathematics, it becomes possible to predict and locate an earthquake (pp. 158–178).

The book is entirely enjoyable when it interrupts, from time to time, the scientific discourse with a small pause, by telling a story, a “once upon a time”, such as that ancient legend told long ago in Japan to explain why the Earth trembled: a gigantic catfish named Namazu lived beneath the Earth, and whenever he shook his tail, the whole world began to tremble (pp. 12–22). In other parts of the book, one also encounters the story of Poseidon, the Greek god of earthquakes, followed by that of Aristotle (pp. 87–117), who believed that earthquakes came from winds trapped beneath the Earth. The book also recounts the story of Zhang Heng (pp. 136–157), an ancient Chinese scholar who designed the first earthquake detector. He created a large vessel surrounded by dragons holding balls in their mouths; when an earthquake occurred, a ball would fall in the direction of the earthquake (pp. 136–157). The book also refers to the

Apollo program missions, which made it possible to install seismographs on the Moon, as well as the InSight seismograph sent to Mars in an attempt to uncover the mysteries of that planet (pp. 224–254). Magnificent stories which show how human beings have always sought to explain or to listen to the invisible.

One also becomes immersed in the accounts and real testimonies reported by people who experienced earthquakes: some compare the sensation to being aboard a boat, others describe the movement as a dance, a rolling of the ground or of the walls. And all this is important, because these perceptions also contribute to understanding the impact and influence of earthquakes, in order to construct a more complete image of the phenomenon (pp. 87–116). Personally, I experienced earthquakes twice in my life, fortunately without damage in my city: the first time in Casablanca, I believe about 2011 or a little later, then another time in Mohammedia during the 2023 Marrakesh–Safi earthquake in 2023, whose epicentre was located near Ighil, in the High Atlas. Even from a distance, the tremors were sufficiently striking to remind us how profoundly the deep movements of the Earth can be silently felt far beyond their point of origin [4].

The book becomes even more appealing when Tkalčić occasionally intersperses the scientific discourse with short explorations of exceptional places, of his exploratory adventures and his scientific journeys, in order to provide a vision of the real life of a geophysicist seismologist (pp. 158–223). He speaks extensively about California and the famous San Andreas Fault, which gave rise to the 1906 San Francisco earthquake. He also devotes a major chapter to the red Australian Outback (pp. 158–186), which he describes as an almost extraordinary world, an ideal natural laboratory for studies in geophysics, situated near the boundaries of tectonic plates where the plates collide intensely. In this universe, every mountain, every rock tells the ancient story of the Earth and of past events, such as Uluru (pp. 158–186).

Scientists install seismometers everywhere in order to listen to the interior of the Earth, and often, even at the bottom of the oceans (pp. 188–222); every place matters and contributes to constructing a more complete vision of the underground world.

The book closes while preserving the spirit of the “Moho”, that invisible boundary which also reminds us of the limits of science itself. Despite all scientific advances, certain regions close to the Earth’s core still remain profoundly mysterious and constitute, according to the author,

the deepest archives of Earth's history. And beyond models, calculations and technologies, the author reminds us with simplicity that the best protection against earthquakes remains, above all, preparedness.

Through the figure of Andrija Mohorovičić, Hrvoje Tkalčić seems to extend this intellectual heritage with the same sensitivity towards the Earth and natural mysteries. Such figures succeed in helping us better to understand our own place in this fragile world, the risks surrounding us, the invisible balances upon which our existence silently depends, and the limits necessary to restrain the excesses and abuses of human beings towards nature. The world today perhaps needs more than ever these rare personalities, such as Birutė Galdikas, Jane Goodall or Wangari Maathai, whose work extends far beyond their scientific speciality in order to become a presence capable of acting in favour of humanity and the entire world.

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