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Full Length Research Paper

Current situation of solid waste management in East African countries and the proposal for sustainable management

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Effects of unsustainable solid waste management are found all around the world but it is worse in developing and under developed countries like East African Community (EAC) countries. A big proportion of their solid waste is not properly managed. This paper highlighted the situation of solid wastes management in EAC countries and compared with other countries. More than 62.5% of generated solid waste in EAC is organic, 19.6% of papers and plastics, 3% of glasses while other kind of waste occupies 14.9%. Waste management (WM) system in developing countries is dominated by insanitary landfill which cover more than 59% of the total collected SW, between 13 and 33% is openly dumped, a negligible quantity is recycled while between 6 and 26% is inappropriately thrown. Only less than 50% of the total generated solid waste (SW) in developing countries is collected and this is the same case in EAC. Sanitary landfill, sustainable composting, waste to energy (WTE) and other recycling system can change waste from unwanted materials to important products. Almost all generated wastes in developed and highly developing countries are collected, with high generation site sorting and sustainably treated and managed. Some countries achieved zero landfilling while others have sanitary landfills. The final destination of each kind of waste in developed countries determine collection mean and improve the quality of raw materials for recycling companies. Value of sorted waste to the recycling companies improves the interest of generation site sorting and maximization collection.

Key words: Solid waste management, East African Community (EAC), waste collection system, future waste management, level of income, waste prioritization, sustainable solid waste management.

INTRODUCTION

Solid waste is one of the big problems all around the world and it becomes even worse in developing and under-developed countries (Scheinberg et al., 2011). This situation is mainly found in many cities of these countries where the main cause is the rapid urbanization, improper city planning and lack of prioritization of SWM by many governments (Aparcana, 2017). East African Community (EAC) is a regional inter-governmental organization of 6 partner states: The Republic of Burundi, Kenya, Rwanda, South Sudan, the United Republic of Tanzania, and the

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Republic of Uganda as shown in Figure 1 (Turner, 2017). All these countries are classified in developing and under-developed countries based on their income levels (UNCTAD, 2018). Living conditions, economy and many other factors affecting characteristics of generated solid waste are similar in EAC. So, the composition of solid wastes generated in these countries is very similar.

In general, the important and basic ways of solid waste management is the control of its generation (reduce waste as much as possible), improved collection system and transportation to the treatment sites (Mbiba, 2014). Smartness of these steps based on waste characteristics and generation rate of community makes treatment easier and quicker (Matsumoto, 2011; Lederer et al., 2017). Like other developing and under-developed countries, a big proportion of EAC solid waste is organic which can be composted easily (Lederer et al., 2017; Das et al., 2019). Studies on composting of organic wastes have been conducted in many countries and it has been concluded as a good alternative on solid waste management especially in developing countries (Couth and Trois, 2012a, b).

For instance, Clean Development Mechanism (CDM) by composting in Uganda started in 2007 and its results are impressive. An experimental study of compost application on beans yield have been done in 8 different sites in Uganda and showed an important increase of yield in terms of quantity and quality. This trial increased the will of farmers on buy and use of compost. However, the quality of produced compost is still critical due to poor sorting, and consequently affects market (Alemiga, 2017). Smart collection and sorting are the key ways of improving waste transportation as big quantity (organic) can be composted at source of generation (Loan et al., 2019). These also result on improving the quality of compost and price reduction which has been found to be a barrier to farmers (Oteng-Ababio et al., 2013; Mbiba, 2014; Isugi and Niu, 2016; Lederer et al., 2017). In contrast, almost all solid wastes generated in EAC are either insanitary landfilled, openly dumped or thrown in inappropriate places, only a negligible quantity is recycled or composted (Okumu-Okot, 2012; Guerrero et al., 2013; Mbiba, 2014; Lederer et al., 2017). In this review paper, different solid waste management systems, sustainable and non-sustainable, challenges and ways to improvement based on experience of developed and highly developing countries will be highlighted. Both sustainable and non-sustainable management in EAC countries will be compared with other developing countries in Africa and with some countries of other continents. The journey of developed and highly developing countries to sustainable waste management will be also reviewed to find the gap between these countries and EAC. At the end of this paper, the best ways to improve solid waste management (SWM) in EAC will be suggested. Suggestion will be based on countries with good history in waste management, EAC economy, and the characteristics of generated waste as well.

METHODOLOGY

Study area

This review was conducted on EAC countries in comparison with other countries. Figure 1 illustrates the map of all 6 EAC countries. This is a home of 177.2 million populations with a density of 80.6 persons/km^2 and a population growth rate of 2.9%. In 2018, the overall EAC real GDP growth rate excluding Burundi grew to 6.5% from 5.9% in 2017 with the highest growth rate of 8.6% from 6.2 for Rwanda while Tanzania, Uganda and Kenya recorded 7 from 7.2, 6.3 from 5.1 and 6.1 from 5.1%, respectively. Report of EAC in 2015 projected population urbanization to increase from 39% in 2014 to 70% in 2050.

Document review

Available published papers, governmental and NGOs reports on SW in EAC were reviewed and used as source of information. Due to scarce publications on EAC waste management, some information has been collected from available papers and compared with other countries with similar living standard. These helped to have some relevant information of SW from generation to final management. These information were compared with highly developing and developed countries to understand their ways to sustainable waste management, difficulties faced, current situation and their future projection. Review was based on the current information and it is divided into four main categories: (1) SW management in EAC countries; (2) solid waste management in other developing countries in Africa and other continents; and (3) SWM in developed and highly developing countries (4) some important innovation that EAC can learn from developed countries. Developed and highly developing countries were chosen according to their sustainable waste management, their route to current situation and their future projection, while other developing countries were chosen based on their past and current situation of SWM, economic status and the characteristics of generated waste.

RESULTS AND DISCUSSION

General characteristics of solid waste in EAC countries

Characteristics of solid wastes generated by a community depend on many factors. These can be due to working conditions, living standard, life style, income level and so on as shown in Table 1 (Marshall and Farahbakhsh, 2013). The characterization of solid waste in EAC's cities showed that a big proportion is organic waste (Okumu-Okot, 2012; Mbiba, 2014).

It is clear that change in living standard affects the characteristics of solid waste generated by a community. From Table 1, it can be seen that a big change appears on organic waste with a difference in percentage of 36 from low to high income community followed by papers with a difference of 26 while other compositions do not show a big change. This means that income level has a high influence on the quantity of organic waste available

Figure 1. EAC map.

Table 1. Worldwide impact of income level on solid waste composition

Income level	Organic (%)	Paper (%)	Plastic (%)	Glass (%)	Metal (%)	Other (%)
Low income	64	5	8	3	3	17
Lower middle income	59	9	12	3	2	15
Upper middle income	54	14	11	5	3	13
High income	28	31	11	7	6	17

in the total solid waste generated by community. Linkage between income level and composition of waste generated by communities (Henry et al., 2006; Oteng-Ababio et al., 2013; Farley et al., 2019) as well as the findings of Mbiba (2014) conclude that as big proportion of EAC's people is found in low and low middle-income level, thus a big proportion of their wastes is organic. It means that a big proportion is decomposable which can be composted easily.

However, poor sorting and collection is a barrier for composting as well as recycling of other wastes like plastics, metals, electronics and so on (Isugi and Niu, 2016). Improved sorting and collection are the basic keys to sustainable waste management by increasing the quantity and quality of waste to be composted or recycled and consequently improve the quality of the end products of recycling. From literature it has been concluded that almost 62.5% of all EAC solid wastes are organic as shown in Table 2; some are easily decomposable which can be composted others are slowly decomposable which can be sanitary landfilled (Oyoo et al., 2014). So, based on today's EAC economy and the cost of some sustainable WM, they are suggested to reinforce sanitary landfill and composting as their main solid waste management system. The remaining 19.6% is plastics and papers, 3% glasses and 14.9% of other kinds of waste can be directly recycled (like metals, paper, plastics, etc.), incinerated and so on.

Research showed that a big proportion of EAC solid wastes are unsustainably landfilled or dumped, another proportion is thrown in inappropriate places while only a very small proportion is recycled (Mbiba, 2014; Aparcana, 2017; Lederer et al., 2017). The main difference between EAC landfills and developed as well as highly developing countries (like China) is sustainability which consider the treatment of landfill leachate and gases (Das et al., 2019; Mishra et al., 2019). Sanitary landfill reduces the risk of leachate ground water pollution as well as atmospheric air pollution. In developed and highly developing countries, composted wastes are characterized by improved generation site sorting which results on increasing the quality of compost produced (Matsumoto, 2011; Farley et al., 2019; Knickmeyer, 2019). Not only these methods but nowadays worldwide environmental researchers are interested on waste to energy through incineration, biological fermentation of organic waste for biofuel production and so on. All these treatment methods show a great contribution in solid waste management because it increases the quantity of wastes treated and the quality of their end products (Weimer et al., 2015; Jankowska et al., 2017; Abdallah et al., 2019).

The dominance of organic waste in EAC can be explained by their industrialization. Look back to the economy, EAC countries are not industrialized. Their

Table 2. Characteristics of solid waste generated in East African major cities.

Waste composition (%)	Dar es Salaam/Tanzania	Moshi/Tanzania	Kampala/Uganda	Kigali/Rwanda	Jinja/Uganda	Lira/Uganda	Nairobi/Kenya
Bio-waste	71	65	77.2	68	78.6	68.7	65
Paper	9	9	8.3	9	8	5.5	6
Plastic	9	9	9.5	5	7.9	6.8	12
Glass	4	3	1.3	-	0.7	1.9	2
Metal	3	2	0.3	2	0.5	2.2	1
Others	4	12	3.4	15	4.3	14.9	14

food processing industries are very few; thus, a big quantity of food consumed is fresh and results in generation of high quantity of organic waste compared to developed countries where a big quantity of their food is pre-processed and generated few organic waste. The reduction of organic waste in developed countries occurs with the increase in paper and plastic waste as shown in Table 1; this is due to the use of papers and plastics for food packaging. While the data in Table 2 show high level of organic waste in some EAC cities, low paper and plastic waste generation can also be explained by low food packaging habit. However, plastic waste shows a big change in some cities comparatively to papers; this is due to restriction of use of plastic as bags and packaging in some countries (Kabera and Nishimwe, 2019). Metals and glasses which are usually found in construction sites need further research to know the reason of change (Isugi and Niu, 2016; Han et al., 2018; Kwori, 2019). Based on worldwide situation of SW, it can be predicted that the quantity of waste will continue to decrease. This must be carefully considered and as the level of income is increasing, the budget of solid waste management must also be increased to improve the current waste management system as well as introducing new sustainable systems (Owusu et al., 2012; Oteng-Ababio et al., 2013).

Solid waste management and control in EAC

SWM is a multistep system and the results of the next step are in function of the previous one. This means that a good management of solid waste must start from its generation to their final disposal or treatment (Kassim and Ali, 2006; Katusiimeh et al., 2012; Oteng-Ababio et al., 2013; Knickmeyer, 2019). As shown earlier, a big proportion of generated SW in EAC is unsustainably managed. This is mainly due to their low income, poor living standards, lack of SWM prioritization by governments, inadequate knowledge on SWM and the effects of its improper management and so on (Das et al., 2019).

Waste generation, collection and transportation

The first step to sustainable SWM is generation control. This is a basic way of WM; it directly reduces the quantity of waste to be generated by a community through maximizing consumption (Matsumoto, 2011; Knickmeyer, 2019; Loan et al., 2019). After generation, waste must be collected; almost all collected solid waste in developing and under developed countries are poorly sorted. Note that the quantity of waste collected is usually less than half of the total waste generated. These wastes are finally transported to landfills, dumped or composting sites while another proportion is thrown to the streets, water bodies and so on (Oyoo et al., 2014). Among all these solid waste management systems, only landfilling and composting can be considered as sustainable and require a careful sorting (Farley et al., 2019). In contrast, almost all EAC landfill are not sanitary; these are only holes used for waste disposal usually open, without compaction or leachate recycling (Komakech et al., 2014; Ephantus et al., 2015; Scarlat et al., 2015). Sustainable Sorting must focus on waste characteristics carefully decomposable and non-decomposable, hazardous and non-hazardous. The best collection system makes further treatment easier because each kind of wastes is treated based characteristics. In EAC and many other African cities, collection system is very poor because it is mostly done without wastes characterization, this cause many difficulties during treatment and increase the cost of sorting (Oteng-Ababio et al., 2013). SW collection system in EAC's countries is considered as informal. This system is mainly done in three steps as follow: community collection, from community collection to transfer point and from transfer point to final disposal. Frequency of

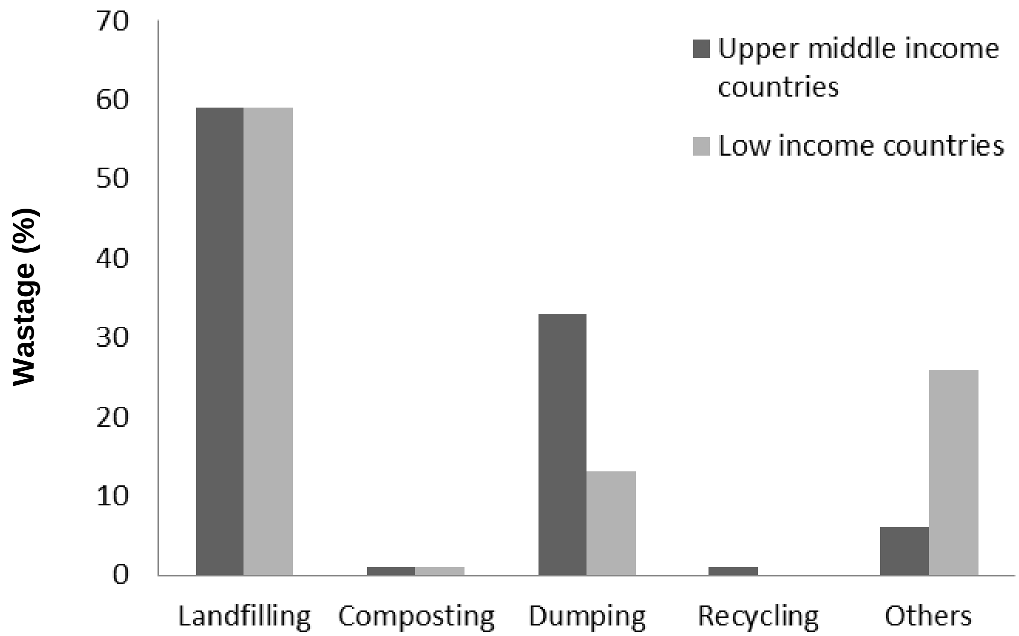

Figure 2. Upper middle-income and low-income countries waste treatment methods.

collection is mainly based on income level of community; the higher the income of community the more the frequency of waste collection and transportation and vice versa (Okumu-Okot, 2012; Sandhu et al., 2017). For instance, in Rwanda, collection is by house to house using different means of collection but the common one is by using plastic bags. Waste is transported to treatment site by companies in charge of waste transportation. The day of waste transportation, plastic bags deposed to the streets are picked by waste transportation companies and transported to the treatment site (Isugi and Niu, 2016; Elias et al., 2017).

In all EAC countries, waste from markets are collected to the nearest transfer points with or without sorting and further transported to disposal site. These cause serious problems because some wastes remain at the street or transfer point. Sometimes waste decomposition start in collection bags or at transfer point due low frequency of transportation, insufficient trucks, quality of roads and so on. These cause bad smell and produce some liquid (like leachate) which result in environmental pollution and cause diseases to the nearest population. Waste transportation has a big contribution on the final treatment. In EAC; transportation is done using open or closed trucks; densely populated cities (urbans) have more trucks than less populated cities. Unsorted or poorly sorted wastes are transported to the disposal sites by trucks and sorting is usually done at treatment site. This affects the efficiency of sorting, cost of treatment through the increase of sorting fees, overloading of trucks and directly affects the efficiency of waste treatment (Kassim and Ali, 2006; Katusiimeh et al., 2012; Okumu-Okot, 2012). Unsustainable solid waste sorting and collection also affect the quality of treatment end products and decrease landfill service life (Mbuligwe, 2002; Lederer et al., 2017).

Waste treatment and recycling

In general, there are different types of solid waste treatment and recycling; a community chooses their favorite based on different factors such as incomes level, types or characteristics of wastes, the main purpose of waste treatment and so on. The common methods of solid waste treatment in high income countries are sanitary landfill, thermal treatment, wastes to energy and so on. Contrary, lower and lower middle-income countries' (like EAC) wastes are unsustainably treated (Bhada-Tata, 2012; Owusu et al., 2012; Mishra et al., 2019). Literature reveals that in low income countries, 59% of their wastes are landfilled, 13% dumped, 1% composted, 0% recycled and 26% unclear. While in upper middle-income 59% of their wastes are landfilled, 33% dumped, 1% composted, 1% recycled, while the remaining 6% are unclear as shown in Figure 2.

All these treatment methods are common in under-developed and developing countries (Mishra et al., 2019). A big proportion of their solid waste is unsustainably treated and result in environmental pollution (Owusuet et al., 2012). From Figure 2, it is clear that a big proportion of solid wastes in these countries are landfilled. A big difference is found only on unclear method, this means that apart from known methods of waste treatment and

Figure 3. Unclear method of solid waste treatment.

disposal there is another proportion of waste which is thrown in inappropriate places. Unclear waste disposal in low and upper-middle income countries are at a percentage of 26 and 6%, respectively. These wastes are improperly managed, either directly deposed in water bodies, to the streets and roads, or to agricultural land as shown in Figure 3 (Mireri et al., 2007). Comparison shows that dumping increased from 13% in low income to 33% in upper-middle income countries. This is not sustainable but explains the relationship between income level and waste management method. In many communities of upper-middle income countries, the level of waste collection is higher than in low income countries; but due to financial issues, inadequate knowledge on SW and lack of SWM prioritization; collected wastes are not properly treated or managed but dumped a bit far from communities or market (Kasmiro Gasim, 2019; Yusuf et al., 2019).

Solid waste management difficulties in EAC

Solid waste treatment and management in EAC countries face many challenges which affect its sustainability. These challenges can be classified based on social, economic and climatic factors (Han et al., 2018). Family size is directly proportional to the daily quantity of wastes generated and composition depends on their economic status, living conditions and so on. In many developing countries (like EAC), cities are densely populated and characterized by poor planning. These affect either directly or indirectly management of solid waste generated; due to improper, insufficient or even total absence of waste management and treatment infrastructures compared to the population of communities they have to serve (Aparcana, 2017; Knickmeyer, 2019). Population growth, size and living standard must be factors to consider during decision of solid waste management. EAC leaders and environmental decision makers must carefully take into consideration rural to urban migration as well as waste generation rate. These facilitate decision making of SWM infrastructure size, numbers, location as well as waste transportation means for sustainable management.

Level of knowledge on waste management is also another factor to be considered. Many people do not have adequate knowledge about solid waste and their impact on life or environment in general. People do not consider their contribution, but they think that every responsibility is that of governments, local leaders or private companies in charge of waste management (Mbuligwe, 2002). The awareness of some local leaders and private companies are also critical. Some are interested in waste management, others are not or are targeting quick income. So, governments, NGOs, experienced private sectors, experienced local leaders and environmentalist must collaborate to organize more training to all level of people to raise their knowledge on solid waste management and improve their contribution in this issue. Culture also challenges the implementation of solid waste treatment and management. Many people think that waste have to be thrown in land for decomposition and become fertilizer or thrown into nearest water bodies and so on (Mireri et al., 2007). This is the culture of many people in developing countries. In many EAC cities, people use to throw waste in rivers during night or when it rains. Primarily for hiding their waste to reduce collection and transportation fees, secondarily because it is their long term behavior and thirdly due to poor collection and transportation mean. Usually people do not accept changes at the same levels; so, trainings on impact of improper SWM and people’s contribution on sustainable waste management must be prioritized (Okumu-Okot, 2012; Marshall and Farahbakhsh, 2013; Aparcana, 2017).

Economy is another challenge on SWM especially in EAC. SWM requires a high investment while direct profit in term of money is low. In EAC, landfilling and composting are considered as their main solid waste management and treatment system. However, due to low investment in this sector most of their landfills are not sanitary, their wastes are not or poorly sorted and the quality of compost is very poor. Low investment does not have effects on the final disposal only but to all steps of waste management. For instance, in many EAC cities the frequency of waste transportation depends on the income of the population. The more the income of communities, the more the frequency of waste transportation (Kirama and Mayo, 2016). This causes a big problem of pollution in low income regions and becomes worse when it is privatized (Sandhu et al., 2017). So, governments must invest more and elaborate clear rules and regulations of solid waste management and create a good working environment especially for private companies. The budget of waste treatment and management depends on the economy of each country; the lower the economy, the lower the budget and vice versa (Han et al., 2018; Das et al., 2019). However, no matter the low investment but good plan from waste generation to the final disposal or treatment can increase the quantity of waste to be managed by EAC communities. These can be achieved through minimizing waste generation as much as possible, improved sorting and collection, wastes recycling and reuse (composting, waste to energy), sanitary landfill and so on. In turn, some money will be gained from this improved waste management to support the budget.

Climatic condition is a factor which mostly affects the quality of roads especially during the rainy season. This directly affects the frequency and the time of waste collection point emptying and transportation. Survey conducted showed that waste disposal areas in many EAC cities are not connected to good roads which cause many difficulties during transportation (Henry et al., 2006). To overcome this, on-site waste treatment must be prioritized focusing on quickly decomposable waste (organic) by composting and other waste can be transported to another treatment place when conditions of roads are good. Administrative factors where some authorities do not prioritize solid waste management and treatment is also another concern (Henry et al., 2006; Okumu-Okot, 2012). All these highlighted challenges will be solved by prioritization of solid waste by authorities and down step by step to local people through trainings and increase their knowledge on the impacts of solid waste, role of waste treatment and their contribution on this issue.

Common EAC solid wastes management and treatment systems (landfill, composting and open dumping)

Like many developing countries, the common SWM system in EAC countries is poor or insanitary landfilling, poor composting and open dumping (Alemiga, 2017; Idowu et al., 2019). In these three management systems, only landfilling and composting can be considered as sustainable. However, they are not, this is due to non-engineered landfills which results on environmental pollution through landfill leachate and gases or incompletely decomposed compost which is usually characterized by some indecomposable materials due to poor sorting.

Landfill

In EAC countries and many other developing countries, many landfills are considered as non-sustainable due to many factors which are usually ignored during landfill site selection, construction, unprofessionalism, lack prioritization of SWM and so on (Owusu et al., 2012). Sanitary landfilling does not only consider waste disposal but also further treatment of landfill leachate and gases from waste decomposition (Idowu et al., 2019; Mishra et al., 2019). In many developing countries (including EAC), more than 59% of all collected solid wastes are landfilled but among all available landfill in EAC only very few are engineered. This results in indirect environmental pollution through underground water pollution by leachate infiltration and air pollution by landfill gases (Isugi and Niu, 2016). Landfilled wastes in EAC are not poorly sorted before disposal; these wastes are composed of organic and inorganic waste. Landfilling of almost all collected wastes as well as unplanned or unexpected waste generation rate result in disturbance of landfill life cycle. Maximization of on-site waste generation sorting increase the quantity of waste to be composted or recycled thus the remaining quantity can be landfilled and consequently reduce the risk of landfill life cycle disturbance (Aparcana, 2017). Based on big proportion of waste which is still dumped or thrown in water bodies, uncollected waste and a big number of insanitary landfill (Henry et al., 2006; Guerrero et al., 2013) efforts are needed in onsite sorting, collection and increase number of sanitary landfills. It has been concluded that there is a direct correlation between sustainable waste sorting and collection and the quantity of waste recycled rather than landfilling (Ferraris et al., 2013).

Composting

Composting is a process which converts biodegradable material such as garden or kitchen waste into a stable material that can be used as a soil improver. This can be considered as the priority option of SWM especially in EAC where about 62.5% of all generated solid wastes are biodegradable (Okumu-Okot, 2012; Oyoo et al., 2014). This is not the only factor which favors the selection of compost as SWM option in EAC, but also agriculture which is their first economic activity. So, composting must be improved as a way of solid waste management and treatment and also as a source of compost for farmers. In EAC and many other developing countries, some composting plant are available but still very few which cover not more than 1% of the total collected solid waste. The low quality of compost produced in EAC affects its market and reduces the interest of investors in composting. So, to improve the quantity of waste to be composted, sustainable on-site sorting needs to be reinforced and prioritized thus improving the quality of compost produced (Couth and Trois, 2012a, b; Isugi and Niu, 2016; Lederer et al., 2017). Sustainable composting increases the quantity of solid wastes treated in developing countries as a big proportion of all generated solid waste can be easily composted even at the place of generation (Loan et al., 2019). This reduces the problem of overloading of wastes transportation trucks and increases the quality and quantity of waste to be recycled (Couth and Trois, 2012a). The use of compost by EAC farmers is a solution to unaffordability of inorganic fertilizers due to high cost which result on low yield in quality and quantity (Isugi and Niu, 2016; Potdar et al., 2016; Lederer et al., 2017).

Open dumping

Open dumping is not considered as a sustainable SWM due to its impact on environment. However, due to different reasons, open dumping is common and occupies the second position of waste disposal mean in upper-middle income countries and the third in low income countries. Wastes are collected from communities, markets and other generation sites without sorting then transported and deposed at a selected open air place. Research showed that in upper-middle and low-income countries, between 13 and 33% of all collected solid waste are dumped (Owusu et al., 2012; Farley et al., 2019). This is the same situation in all EAC countries which are also classified in this category of incomes. Review showed that from low to upper-middle income countries, dumped wastes increased from 13 to 33% while unknown management decreased from 26 to 6% of all collected SW (Bhada-Tata, 2012). This shows that, despite its effect, open dumping is legally accepted in these countries and can divert a big proportion of unknown management to dump (Waweru and Kanda, 2012; Kasmiro Gasim, 2019). However, open dumping causes a serious problem of environmental pollution; either directly or indirectly. This becomes worse to the population around these dumping sites due to runoff into water bodies, bad odors, attracting flies and breeds, soil pollution, reduction of soil infiltration rate and so on (Mireri et al., 2007; Okumu-Okot, 2012). As open dumping is not sustainable, waste to energy, sanitary landfill and composting must be prioritized to reduce secondary pollution from dumping and produce important products from these wastes.

Insanitary landfill, incomplete composting and open dumping are serious problems in all EAC countries.

These cause pollution to the surrounding environment; the effects can be either direct or indirect and the routes of exposure differ accordingly. This pollution also affects different activities of EAC people like agriculture which is their first economic activity (Mireri et al., 2007; Oyoo et al., 2014). Improper solid waste management affects agricultural yield through soil and water pollution, this can be caused by landfill leachate and gases, indecomposable waste (like plastics, and metals) and long-time decomposable wastes which affect soil infiltration rate and so on (Mireri et al., 2007). Open dumping also attracts flies, breeds and other disease vectors which cause health problems to the surrounding population, pests and diseases to crops, and so on (Okumu-Okot, 2012). So, as population is growing quickly and directly proportional to solid waste generated, this waste must be prioritized by all governments, NGOs and people as well for protecting environment.

SWM in other developing countries

The situation of solid waste management in other developing countries across the world is not far away from that of EAC. The characteristics and management of SW differ according to the economy of each country and the quantity of waste managed increase with increase in economy. The composition of SW in developing countries can differ from one country to another but in general organic waste dominate all over the world while other composition can differ according to different reasons (Das et al., 2019; Perteghella et al., 2020). A case study in 8 least developed countries in Asia (Afghanistan, Bangladesh, Bhutan, Cambodia, Laos PDR, Maldives, Myanmar and Nepal) showed that the composition of their solid waste is dominated by organic waste. The level of organic waste varies according to the country but in general it ranges between 30 and 70%. Plastics and papers also fluctuate according to population living condition with a very big change on plastics waste which is usually caused by measures of each country for their rules of restricting the use of plastic bags, but in general the range is between 10 and 50% (Glawe et al., 2005; Vazquez et al., 2020). These compositions fall in the same range with EAC countries.

Many developing countries have been reported to have a big number of population who rely on money from waste picking at disposal sites. This is considered as the common method of waste sorting in these countries. Collected wastes are transported by companies and deposited at selected places, usually open-air dumping or insanitary landfill as shown in Table 3. Waste pickers, usually women and children sort these wastes at deposition site, not for sustainable waste management but for selecting waste which can be sold to the recycling

Table 3. Comparative summary of SWM between EAC and other countries.

Economic status	Country	Waste composition (%) Organic	Papers and plastics	Glasses	Others	Waste Management system (%) Landfill	Composting, recycling and Incineration	Open dumps	Others	References
Developed and highly developing countries	Germany	30	37	10	23	0	100	0	0	Mühle et al. (2010) and Pomberger et al. (2017)
	UK	38	25	7	27	57	39.7	0	0	Patrick (1985); Mühle et al. (2010), and Wang et al. (2020)
	Belgium	35	38	5	22	0	100	0	0	Gentil (2013), Pomberger et al. (2017), and Sharma and Jain (2020)
	China	58.8	20.5	5	15.7	63.7	36.3	0	0	Liu et al. (2017) and Duan et al. (2020)
	India	51	17	-	32	93*	7	0	0	Malav et al. (2020)
	Italy	35	30	6	29	34	66	0	0	Ferraris et al. (2013), Pomberger et al. (2017), and Ripa et al. (2017)
Developing countries	Bangladesh	74.5	12.6	0.8	12.1	86.5*	13.5	0	0	Shams et al. (2017), Islam and Moniruzzaman (2019), and Alam and Qiao (2020)
	Algeria	64.6	26.4	2.8	6.2	0.2	2	96.8	1	Guermoud et al. (2009), Naïma et al. (2012), and Scarlat et al. (2015)
	Cameroon	70	16	4	10		5	95	0	Scarlat et al. (2015) and Sotamenou et al. (2019)
	Niger	57	35	2	6	64*	4	-	32	Oumarou (2015) and Scarlat et al. (2015)
	Thailand	65	27	-	8		11	-	-	Tuprakay et al. (2014)
	Bulgaria	64.3	16.5	4.4	15.8	74	26	0	0	Barata (2003), Inglezakis et al. (2012), and Pomberger et al. (2017)
EAC countries	Kenya	65	18	2	15	75*	9	16	-	Henry et al. (2006), Gakungu et al. (2012), Waweru and Kanda (2012), Mugo et al. (2015), and Palfreman (2015)
	Uganda	75	15	1	9	41*	8	51	-	Komakech et al. (2014) and Yusuf et al. (2019)
	Rwanda	68	14	-	17	79*	10	11	0	Isugi and Niu (2016) and Kabera and Nishimwe (2019)
	Tanzania	68	18	4	8	60*	10	30	-	Sharma and Jain (2020)
	S. Sudan	35.5	33	4.5	27	-	-	100	-	Cowling (2013), Kasmiro Gasim (2019), and Mohamed and Elhassan (2019)

companies (Ahmed and Ali, 2004; McBean et al., 2005). These people are vulnerable due to improper protection from risks which can be caused by hazardous waste. Note that almost all facilities and infrastructure for waste management are found in capital cities and secondary cities while there is no single waste management infrastructure in rural areas. The frequency of waste transportation varies from capital cities to secondary cities; high frequency in capital cities is supported by big investment which results in having many trucks and good roads while secondary cities invest less and their roads are not good enough (Al-Khatib et al., 2007; Olay-Romero et al., 2020). No matter the investment or waste transportation facilities, but a big proportion of waste is disposed in insanitary landfill or in open dump. Review shows that landfilling is leading all systems of waste management in developing countries. This is dominated by insanitary landfills with poor site selection and planning which results in ground water and air pollution. The lifecycle of these landfills is usually unpredictable due to unplanned increase of waste generation, disposal of all kind of waste without sorting, lack of compaction as well as rapid population growth.

Open dumping which is ranked the second receiver of collected wastes in developing countries is not environmentally friendly but it can be legal or illegal. It is legally accepted when in charge of waste management agree with leaders to select a specific places of waste disposal. It is also classified as illegal when it is chosen by people themselves (Al-Khatib et al., 2007, 2010; Kasmiro Gasim, 2019). Landfills and open dumps cover almost all waste in developing countries with a small quantity recycled. Note that although the quantity of sanitary landfilled or recycled waste increase with economic growth of the country while dumped quantity decrease; almost all cities in developing countries face the challenges of inadequate or insufficient waste management and transportation facilities (Ahmed and Ali, 2004; Scarlat et al., 2015; Turcott Cervantes et al., 2021). It is also important to know that, not all generated wastes are collected, but there is another big proportion which are not collected. In some cities, collected wastes are even less than half of total generated waste, for instance in Kabul, Afghanistan only 23% is collected. Uncollected wastes are either openly burned, thrown in water bodies, streets, forests or farms which expose population to health problems (Glawe et al., 2005; Sotamenou et al., 2019). Organic waste composting and other waste recycling are also available in some cities; however, these are very rare due to lack prioritization of these sustainable methods by governments.

SWM in developed and highly developing countries

Many criteria are considered for classifying a country as developed or developing. As explained previously the budget of waste management depends on the economy of each country. Contrary to least developed and EAC countries, developed and highly developing countries show a significant difference in waste composition, management, prioritization and investment. Organic wastes are still ranked the first composition of municipal solid waste but it is low compared to developing countries. Papers and plastics are the second while glasses are the third. While plastics are experiencing a quick reduction due to restriction of use of plastic bags; other waste like metals, E-waste and textiles are few but much more compared to developing countries (Srivastava, 2016; Maria et al., 2020). The reduction of organic waste in these countries is due to their industrialization which directly cause a significant difference of other kinds of waste compared to developing countries (Wang et al., 2020).

Over increase of solid waste is a challenge to all countries across the world, but management and treatment is experiencing a big difference between developed and developing countries. There is a big difference from waste generation to the end use; for instance, in 2015, 48.9% of waste generated at Umbria, Italy was sorted at generation site. Recyclable wastes are transferred to recycling while organic wastes are composted at composting sites (Maria et al., 2020). Despite population growth, law of waste prevention helped Nottingham, England to achieve waste reduction from 123,615 tons in 2006/2007 to 115,170 tons in 2016/2017 (Wang et al., 2020). This improved waste collection and reduction lead to sustainable transportation to the final treatment.

Usually, the frequency of waste transportation depends on the characteristics of waste. Time interval of organic waste transportation is shorter than other kinds of waste, this reduces the risk of decomposition at collection site. Organic wastes are composted at designated composting site thus produce organic fertilizers as well as biogas. Recyclable inorganic wastes are also transferred to recycling companies with a long-time interval of transportation frequency. All these developed methods lead to almost 100% waste collection, diversion of waste which could be landfilled at a range between 40 and 80% of the total generated waste in European Union. Diverted wastes are well managed by energy recovery as well as production of other valuable materials (Srivastava, 2016; Pomberger et al., 2017; Wang et al., 2020). Like developing countries, budget of waste management in developed and highly developing countries also depends on the economy of each country. In developed and highly developing countries, this budget is distributed from waste generation to the final disposal. Onsite sorted wastes are sold to scavengers or directly to the recycling companies which also produce other valuable materials. This increases the will of local people on waste sorting (Mühle et al., 2010; Fei et al., 2016). Contrary to developing countries, facilities of waste management in developed and highly developing countries are available in big cities as well as in small cities or even in some rural areas. Table 3 shows that while developing countries are still straggling with insanitary landfills, open dumping and inappropriate disposal, some developed countries achieved zero landfills while others have sanitary landfills(Mühle et al., 2010; Pomberger et al., 2017; 'Eurostat Regional Yearbook, 2018, 2019).

Insanitary landfills and open dumps are still covering almost all collected solid waste in EAC countries. While in developed countries almost all quantity of waste is sorted at generation site before collection and treatment; in EAC and other developing countries, wastes are collected with a very poor sorting. The final unsustainable disposal of these wastes causes a serious health problem to the population near disposal site and environment in general. Note that the data shown in Table 3 illustrate the general characteristics of solid waste in the listed countries. For EAC and other developing countries, only data of urban areas have been highlighted. This is due to lack of enough information of solid waste management in these countries and lack of waste prioritization which abandon rural areas. As shown in the table, percentage of landfills in some developing countries is marked by (*), these landfills are classified as insanitary without gases recovering or leachate recycling and poor site selection. Some countries use open holes without even compaction and consider them as landfills while others confuse landfills and open dumps. According to the characteristics of sanitary landfills, these are not sustainable (Ahmed and Ali, 2004; Scarlat et al., 2015; Kasmiro Gasim, 2019;

Alam and Qiao, 2020).

GOVERNMENT AND PRIVATE SECTORS' CONTRIBUTION ON SOLID WASTE MANAGEMENT IN EAC

Almost all treatment and management of solid waste in EAC countries are in charge of governments; only a small part is on the hands of private companies (Kirama and Mayo, 2016). Each side has its contribution but also some challenges either due to working principles and conditions, level of income or to their main purpose as shown in Table 4. Low profit (in terms of money) from solid waste management and treatment and lack of prioritization by many governments are the key factors affecting the investment of private companies in this field (Katusiimeh et al., 2012). While the direct profit of solid waste management in terms of money is very low, all private companies invest in this field targeting money. These affect the whole processes of solid waste management and treatment in poor communities thus resulting on focusing in urban areas where income is high (Kassim and Ali, 2006; Isugi and Niu, 2016; Lederer et al., 2017).The decisions of solid waste collection and management by private companies is under the rules and regulations of governments. A big challenge is the implementation of these rules by companies and the follow up of the governments. So, governments must do their best to evaluate the implementation of rules and regulations of solid waste management by private companies and encourage them to work in all regions of countries (low and high income).

Awareness of EAC people on the importance of solid waste management

Awareness of EAC's people on the management and treatment of solid waste is very critical. The causes of these poor understanding are different but can be grouped as follow; lack of SWM prioritization by governments. This is a big challenge which causes total failure of almost all projects of SWM. Life change with new living standard in the past, EAC countries did not consider SW as a problem, but nowadays it is a big challenge due to population growth and change in living condition. So, solid waste must be taken as a priority by authorities and train people to raise their knowledge on this issue. Knowledge of local leaders on SWM will help to overcome the risk of failure of projects of solid waste management and treatment because there will be a common understanding with people (Mbuligwe, 2002; Henry et al., 2006; Okumu-Okot, 2012; Isugi and Niu, 2016). Illiteracy and economy, in many EAC countries, solid waste management is the responsibility of government and this causes a big problem of carelessness of SWM infrastructures by people. This is mainly due to lack of adequate information on the importance of those infrastructures to the surrounding population (Guerrero et al., 2013).

Income of EAC is also another challenge because of poor and unstable resources. This affects prioritization of budget of SWM and results in dumping or inappropriately throwing a big percentage of their wastes. It also causes many difficulties to private companies which rely almost 100% on money (waste management fees) from people they serve (Katusiimeh et al., 2012; Kirama and Mayo, 2016; Sandhu et al., 2017). This causes unaffordability of many people (especially poor people) to the service due to high cost. It also results on throwing their waste in water bodies, streets, open air places and so on. However, some money can be gained from advanced waste management (composting, sanitary landfill through gas recovery, incineration, waste to energy and so on). To overcome these; governments are requested to increase budget of waste management and prioritize recycling than disposal (Katusiimeh et al., 2012). This is a key solution to success of SWM projects through the reduction of cost of handling and always relying on people. Especially on waste to compost, this is an important solution which will provide enough compost to EAC farmers. Compost is an important fertilizer on soil remediation (bio-remediation); erosion control through improving soil water holding capacity and it is also very cheap compared to chemical (inorganic) fertilizers. So, the problem of awareness of EAC people on the importance of management and treatment of solid waste will be solved together by local government, private companies and NGOs through training people to raise their knowledge on SWM (Henry et al., 2006; Okumu-Okot, 2012).

Sustainable waste management provides many opportunities as it is shown in Table 5, there are many possibilities of turning wastes to important products based on experience of developed and highly developing countries. In China, wastes are sustainably managed through recycling, composting, and waste to energy (WTE) and sanitary landfilling. Clear plan, ambition and growth of economy show a remarkable positive change in sustainable waste management especially WTE. European countries, US and Japan are three first producers of energy from waste for long time; however, the rapid growth of Chinese economy and their interest in WTE ranked this country to the 4th position in 2012. In 5 years, from 2007 to 2012, Chinese WTE plants increased from 66 to 100 and since the beginning of 21st century, the quantity of solid waste transformed to energy increased from 2 to 14 million tones. These rapid increases show relationship between economy and waste management system (Dong, 2011; Abdallah et al., 2019; Sharma and Jain, 2020). Economy of EAC countries is a big obstacle to the implementation WTE system, but characteristics of their solid waste and the will of different sectors show other opportunities which can

Table 4. Some key factors affecting SWM in developing countries.

Government	Private companies
Lack of people awareness	Lack of people awareness
Lack of enough equipment and infrastructure	Lack of enough equipment and infrastructure
Investing without benefit (in terms of money)	Waiting for more benefit (in terms of money)
Climatic conditions	Climatic conditions
Illiteracy of people	Illiteracy of people
Improper housing	Improper housing
-	Focusing in high income people

Table 5. SWOT (Strengths, weaknesses, opportunities and threats).

Strengths	Weaknesses
Will of different persons on solid waste management	Many solid waste management projects focus in high income communities
Rules and regulations of environmental protection	Some in-charges of environmental protection are not specialist.
Availability of environmentalists in all EAC countries	Lack of organization
Investors and private companies in inorganic and organic solid waste recycling	Unsustainability of the available solid waste management system (landfills and composting)
-	Lack of enough information on SWM in EAC people
Opportunities	**Threats**
High percentage of organic waste	Unplanned changes in leadership
Big market of compost	Insecurity
Good climate for small scale and local composting	Corruption
Willing of private companies to invest in solid waste management	Illiteracy
Will of governments on prioritization of SWM	Climatic conditions
Enough market of energy from solid waste treatment	Lack of enough infrastructures and equipment
-	Economy

solve this problem. Generated solid waste in EAC is dominated by organic waste; so, sustainable composting can cover a big proportion of their waste. Based on the quantity of waste dumped or unsanitary landfilled in EAC, reinforcement of sanitary landfill is also needed to reduce the risk of secondary pollution. Some pilot project of WTE must also start; so, from the increase in economy and WTE experience; future change of the reduction of waste composted or landfilled to WTE will be achieved.

Conclusion

This paper summarized the situation of solid waste management in EAC countries and analyzed their problems. It is found that income level determines the characteristics and management system of waste generated all over the world. High cost of waste management tends to be a barrier in EAC countries and results in environmental pollution from insanitary landfill, dumping, unsustainable composting and direct disposal of waste into inappropriate places. Unstainable waste management in EAC starts from waste generation to the final disposal due to poor sorting and collection. More than 62.5% of EAC solid waste are organic; these waste can be sustainably composted and produce quality compost to EAC farmers. Sustainable composting and sanitary landfill will result on diverting dumped and inappropriately disposed waste to landfill and composting. Experience of developed and highly developing countries showed that WTE is more sustainable and increase with economy. EAC countries should start pilot projects of WTE and make it a future priority with the economic growth.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Knowledge and attitude of *Khat* growing farmers on the safe use and handling of pesticides in Haromaya Wereda, Oromia Regional State, Eastern Ethiopia

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The awareness level of the farmers growing *Khat* regarding the safe use of pesticide was investigated in this study at Haromaya Wereda, Eastern Ethiopia. In order to achieve the proposed objectives, structured questionnaires, oral discussions, and field observations were organized with the farmers growing *Khat* on the safe use of pesticide. After the study sites were selected purposively, systematic random sampling of the target population was applied. The study result revealed that the farmers lack training on pesticide practices, and most of them use their own experience and consult their neighbours. The findings of the study further indicated that majority of the farmers do not believe that pesticides can be detected in the soil, fruit and leaves of tree, air, ground water and food. Nearly half of the farmers were aware of the harmful effects of using pesticide on insects and birds. The farmers have poor understanding of the effect of long term exposure to pesticides compared to short term exposure. The farmers' understanding of exposure to pesticides through respiratory system and mouth is relatively satisfactory compared to the dermal exposure. Over one third of the farmers had no knowledge of how to store pesticides in the right conditions. However, greater percentage strongly believes that pesticides should be kept out of the reach of children. Even though, majority of the respondents indicated that DDT was used on *Khat*, only few of them have knowledge of its illegal importation. The outcomes of the study confirmed the need of training on the safe use of pesticides to raise the awareness of the farmers and safeguard the health of the farmers and the environment.

Key words: Knowledge, attitude, pesticide, environmental, health, *Khat* growing farmers, Haromaya, Ethiopia.

INTRODUCTION

Pesticides are widely used chemical compounds in agriculture to destroy insects, pests and weeds. In modern era, they form an indispensable part of agricultural and health practices (Esteban et al., 2020). Pesticides have helped to duplicate food production during the last century, and the current need to increase food production to feed the rapid growing human population mounts pressure on the intensive use of

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pesticides and fertilizers (Fernando, 2017). Exposure to chemical pesticides is one of the most significant occupational risks among farmers in the developing countries as they can easily get in contact with pesticides, for example, when mixing the chemicals or when applying them to crops and when pesticide residues are applied in houses (Chitwan et al., 2017). Besides their agricultural uses, pesticides are also becoming essential in household pest control. The continuous application of insecticides has caused some insects to develop resistance. Besides, the continuous use of synthetic insecticides causes ecological disturbance by acting on non-targeted insects (Azeem et al., 2019).

Ethiopia is a developing country and majority of its population, estimated to be 80%, solely depend on agriculture. Reports of United Nations, Department of Economic and Social Affairs (2017) indicated that, the population of Ethiopia was 18,128,000 in 1950 and has grown to 104,957,000 in 2017, and is expected to escalate to 139, 620,000 and 190,870,000 in 2030 and 2050, respectively. Feeding large population size will be one of the great challenges to human kind, and may be one of the most intimidating encounters facing the world during the remaining years of this 21st century (Fróna et al., 2019); thus to feed this rapidly growing population of the country, agricultural yields need to be increased. In addition to this the introduction of high production of fruits, vegetables, horticulture farms, and cash crops is highly pronounced in modern agricultural practice. Due to the frequently changing weather conditions, agricultural practices are facing unpredictable challenges resulting in food shortage and food self-sufficiency. Thus, various strategies are designed towards combating the problems of food security, and increasing food production that can meet the demands of the growing population. Agrochemicals have enabled more food production during the last century, and there is a current need to increase food production to feed the rapid growing human population (Carvalho, 2017).

One of the cash crops, produced in largest quantity in Ethiopia is *Khat*. It is an evergreen perennial shrub plant that belongs to the Celastracene family. *Khat*, a small flowering bush native to the Horn of Africa, is an illegal drug in most countries in Europe, Asia, and North America. It is cultivated in most regional states of the country, though the extent may vary. The potential areas where *Khat* is grown are found in the eastern highlands of Ethiopia. There is a production and prevalence of *Khat* chewing variation among regions in Ethiopia (Awell et al., 2016). The highest *Khat* chewing prevalence is found in Harari (53.2%). Similarly, extensive *Khat* production is the major agricultural practice in all the districts of East Hararghe Administrative Zone of the Oromia Regional State, Ethiopia. The region alone constitutes 53.4% of the total production area of *Khat* production in Ethiopia (Hurni et al., 2016).

According to Mekuria (2018), *Khat* is the third largest export commodity after coffee and oil seeds. *Khat* can generate considerable amount of revenue at individual, household and national level. Some farmers prefer *Khat* to other crops for several reasons. *Khat* is a cash crop, which can bring substantial returns. The plant is less vulnerable to drought with less cost for labor demand throughout its production. *Khat* has both positive and negative economic advantages. On the positive part, it serves as employment opportunity and source of income in the cultivation and marketing processes. The source of cash income (up to 76.8% in Harar) and drought resistance behavior of the plant are among the main economic advantages of *Khat* growing.

Frequent use of pesticides is becoming a common practice by the plant cultivars in order to control the pests, insects and diseases that are significantly affecting the yield. Results of interviews and questionnaires showed that majority of farmers in Chiro Woreda use DDT and other unknown pesticides to grow their *Khat* and majority of them mix DDT and other pesticides, especially malathion (Derso and Dagnew, 2019). Another study also confirmed that some selected pesticides such as aldrin, dialdrin, BHC, diazinon, DDT, 4,4-DDE and heptachlor were investigated in water and *Khat* samples that were collected from five different sites Haromaya Wereda. Diazinon and DDT levels in *Khat* sample one (0.0323 mg/L), *Khat* sample two (0.0293 mg/L) and *Khat* sample one (0.0134 mg/L), *Khat* sample two (0.0173 mg/L), respectively are above the maximum residue level (Adamu et al., 2019). Pilot study participants (retailers and consumers) confirmed that pesticide chemicals are sprayed on *Khat* bushes and trees, and *Khat* farmers disclosed the use of Dichlorodiphenyltrichloroethane (DDT) and Malathion on their farm (Beyene et al., 2020). Another survey study in Haromaya Wereda also identified that the farmers were using pesticides including DDT for growing *Khat* (Regassa and Regassa, 2018).

If improperly used, pesticides can lead to secondary pest outbreaks, destruction of non-target species, soil, water, and air contamination, and residues in primary and derived agricultural products that endanger both the environment and human health (Azeem et al., 2019). Farm workers' exposure to pesticides has been associated with adverse health effects like cancer and birth defects resulting in hundreds of fatalities, the majority of which occur in developing countries (Damalas and Koutroubas, 2016). Farmers and especially those directly involved in the handling of pesticides, are at a high risk of exposure to pesticides through contact with pesticide residues on treated crops, unsafe handling, storage and disposal practices, poor maintenance of spraying equipment, and the lack of protective equipment or failure to use it properly (Kosamu et al., 2020). Pesticide handlers are regularly involved with various activities like mixing, loading or application of pesticides.

Consequently, high care has to be taken during preparation, transportation, storage and handling of pesticides (Ndayambaje et al., 2019). People who work with these chemicals need to receive proper training on the safe use and personal protective equipment to minimize exposure and reduce health risks.

Farmers' knowledge of pesticides and their safe use are critical for implementing effective pest management program. These risks may be exacerbated by lack of information on pesticide hazards, the perception and attitude of farmers regarding risk from pesticide exposure, lack of education and poor knowledge and understanding of safe practices in pesticide use, including storage, handling and disposal (Mustapha et al., 2017). Higher levels of education give pesticide users better access to information and more knowledge of the risks associated with pesticides, and how to avoid exposure. Less educated farmers may be hampered in their ability to understand the hazard warnings on pesticide labels, how to avoid exposure, and how to follow recommended safety and application guidelines. For example, illiteracy and lack of knowledge on the extent to which pesticides represent a hazard have been considered the most important barriers to the adoption of self-protective behaviors by farmers. A higher proportion of pesticide poisonings and deaths occur in developing countries where there are inadequate occupational safety standards, lack of use of personal protective equipment (PPE), inadequate hygienic facilities, illiteracy, and insufficient knowledge of pesticide hazards (FAO and WHO, 2020; OECD/FAO, 2016).

The use of pesticides, for obviously beneficial reasons in *Khat* production, may lead to undesirable effects on the environment and health. Several previous researches identified that farmers in developing countries are exposed to toxic chemicals due to lack of technical knowledge on toxicity levels of pesticides and safety measures to protect themselves from the exposure (Beyene et al., 2020). To make a balance between the beneficial and adverse effects of pesticide use for *Khat* production, investigation of the awareness, knowledge and perception of the farmers on the health and environmental effects of pesticide uses and handling is needed; the aspect that has never been considered in the eastern part of Ethiopia (major *Khat* producing area). Therefore, the objective of this research work is to investigate the knowledge and perception of *Khat* cultivating farmers regarding the safe use of pesticides, proper handling and pesticide storage, and the impacts of pesticides use on human health and the environment.

MATERIALS AND METHODS

The study areas

The survey study was conducted at Haromaya Wereda, East Hararghe Zone, Oromia Regional State of Ethiopia (Figure 1). The district is known for its extensive cultivation of varieties of *Khat* plants and there is a great dependence of the people on *Khat* crop. The *Khat* products are highly demanded both in national and international markets, particularly the one with the local brand name "*Awaday*". It was also observed that losses due to pests, diseases and weeds are the major factors affecting their yields, directly or indirectly. Realizing all these and other related problems, the district was selected as a research site and thus considerable attention was paid to the extent to which the villagers of the study areas understand the risks caused by traditional way of pesticide use and handling.

Data collection

In consulting and discussing with the district agricultural staff, the villages under study were clustered into five mutually exclusive geographical zones to make the data collection systematic and easy to handle within a reasonable period of time. Depending on the size of the clusters, seven villages were sampled from a total of 33 in *Awaday* district (Figure 1). Multistage sampling method was used to prepare an exhaustive list and sampling frame of all members of the *Khat* growing farmers with the help of the agricultural development office. After determination of the sample size, the number of *Khat* producing households, sites and elevation factors (quotient between the size of the population and the size of the sample), the first unit was selected randomly. Then, systematic sampling was applied to draw samples at regular interval from the list. Accordingly, a total of 245 farmers were selected randomly from these villages. Representative number of agricultural workers and health professionals were also made to participate in the interview to evaluate the training status of the farmers on the safe use, handling and alternative use of pesticides.

Questionnaires were developed in English language and then translated to Oromo language (the local language) so that it was easy to communicate with the respondents during data collection. Illiterate respondents (unable to write or read) were assisted by the researcher in reading questions, and writing their ideas. Prior to the data collection, the questionnaires were pre-tested on limited respondents, 20 farmers, and four experts from the health sector and agricultural staff who were living and working in the study areas. In addition to the structured questionnaires, the data collection was also supported by semi-structured interviews and observations in order to validate results of the study.

Statistical data analysis

All the data collected were coded, entered and then analyzed using the Statistical Package for Social Sciences (SPSS) computer software version 20 windows. Descriptive results were expressed as frequencies and percentages for the categorical variables.

RESULTS

Socio-demographic characteristics of the participants

Table 1 reveals that the ages of the respondents who participated in the structured interview ranged from 21 to 61. However, 66.93% of the farmers were in the age range of 30 to 40, which indicated that majority of them are productive. Analysis of the educational level also revealed that 33.1% of the respondents were at no schooling level, illiterate. However, nearly half of the respondents (48.97%) attained basic education (reading

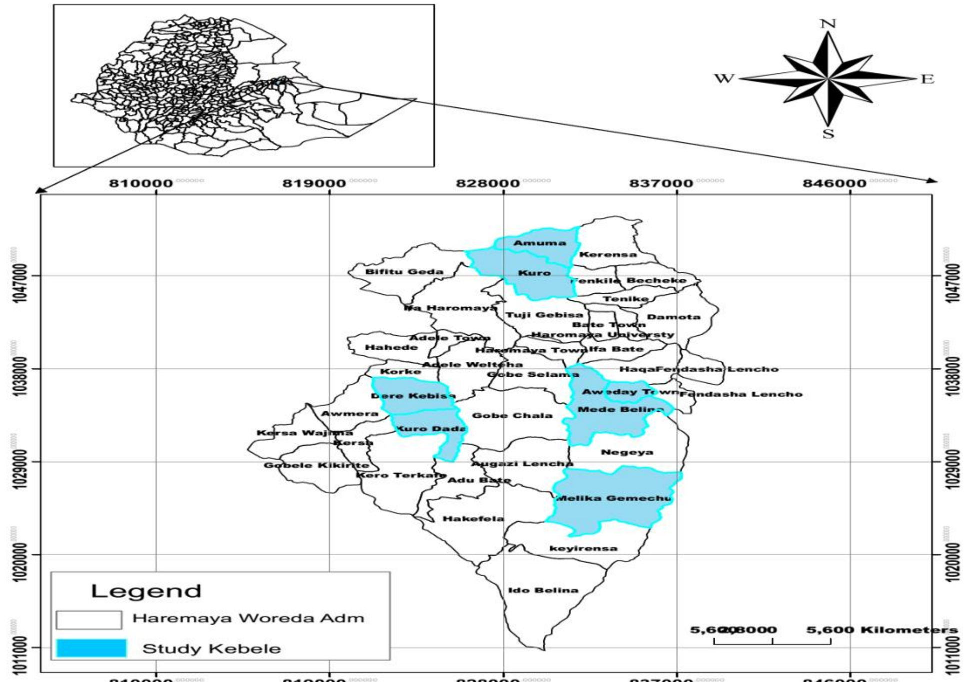

Figure 1. Study sites around the *Haromaya*. The seven study sites were selected including the Amuma, Kuro Finkile, Dere Kebiso, Kurp Dada, Mede Gemechu, Melka Belina and Awaday villages, based on the quantities of *Khat* production.

and/or writing using either the local or national languages). Furthermore, respondents with primary, secondary and diploma levels accounted for about 10.3, 6.2 and 2%, respectively. The research results further indicated that there was no participant with Bachelor degree and above. Almost all (96.7%) of the participants were married and are thus heads of their families. About half (53.1%) of the respondents had household family member that ranges from 6 to 10; and nearly half of the family members were in the age range of 6 to 10. On the other hand, majority of the respondents (95.3%) lived in the study area for more than six years and about 93.2% worked on *Khat* crop for more than six years and thus had rich experiences on *Khat* farming. Demographic characteristics of the *Khat* growing farmers who provided their responses for the study questionnaire or took part in the interview are shown in Table 1.

Pesticide knowledge and perceptions

All the results presented here and the relevant discussions provided subsequently are based on the numerical information compiled in Table 2.

The respondent farmers were asked whether they know or not the name of pesticides they are using on the *Khat* plant. Based on their responses, 63.7% knew the name of pesticides they were using. They were also asked whether or not they knew well about the safe use of pesticides they were using. Accordingly, 42% of the farmers responded that they have knowledge of the safe use of pesticides they use on their farms. Similarly, significant number of the respondents (40.2%) said that they have awareness on the proper storage of the pesticides.

A large proportion (80.0%) of the farmers also responded that they consult their neighbours. However, it was only 16.7% farmers who said that they consulted agricultural experts on the use of pesticides and the corresponding safety considerations. It was also learned that 78% of the farmers used their own experiences when dealing with pesticides for various purposes and handling of pesticides compounds generally. Most of the respondents also said they do not get information from sellers. Only 7.3% of the respondent farmers agreed that they received information, such as brochures, when they were purchasing the pesticides. It was also learned that 95.5% of the sampled farmers used pesticides with instructions in a language they do not understand and only 4.5% understand the instructions for pesticides use. Only few farmers, 12.6%, replied that they read the instructions on the labels of the pesticide containers. On the other hand, majority of the respondents expressed that the information available on the containers were not important.

Besides, the farmers were all asked whether certain trainings were done on proper handling and application of pesticides on *Khat* or other plants. It was learned that on

Table 1. General information and personal particulars of the respondents.

Variable	Frequency	%
Ranges of age (years)		
18-30	24	10
31-40	164	66.9
41-50	31	12.7
51 and above	26	10.6
Educational level		
No schooling	81	33.1
Read and write (Basic education)	120	49
Primary education	26	10.6
Secondary education	14	6.1
College diploma	5	2
Marital status		
Married	237	96.7
Divorced	7	2.9
Widowed	1	0.4
No of family members		
Less than 2	19	7.8
2-5	59	24.1
6-10	130	53.1
11 and above	37	15.1
Stay period in the study area		
5 and bellow 5 years	9	3.7
6-10 years	72	28.8
Above 10 years	164	65.6
Working duration on Khat Farming (years)		
5 and bellow 5 years	17	6.9
Between 6-10 years	170	69.6
Above 10	58	23.7

average, 95.9% of the respondents said that no scheduled trainings were held or delivered on the practices of pesticide usage. However, the remaining 4.1% responded that they received informal training from pesticide retailers on how to operate the spraying devices. It was also known that the explanations by the retailers were simple orientations, when they visit the retailers' shop to purchase the spraying equipment. The respondents were not sure whether the referred retailers were properly trained or not. The subjects were also asked whether they need additional training or not on the safe use and storage of pesticides. The need for further training was stressed by 63.7% of the respondents on safe use and similarly 41.6% on storage of pesticides.

Agricultural experts and health professionals were also asked for their views on the need of training or a program to raise awareness among the communities and households on safety issues related to pesticide use on *Khat* plant. Their reactions to the questions are summarized as follows: out of 21 agricultural professionals, 90.5% expressed that there were no training or awareness raising programs that have been organized and delivered; while 9.5% responded that they have never had any information on the issue. In the same manner, 60% or 20 health professionals also responded that no training or awareness raising programs have been carried out with the subjects while about 35% answered that they did not have any information whether any training was given to the farmers during their stay in the villages.

The respondents were further asked to reflect their opinions on the health effects of the chemical pesticides

Table 2. Study results on the knowledge and awareness of farmers on pesticide uses.

Variable	Frequency	%
Farmers that have enough information on right use pesticides	103	42
Farmers that have enough information on right storage of pesticides	99	40.2
On the right use and storage of pesticides (sources of information)		
From retailer	16	6.5
Agricultural worker	41	16.7
Health extension	16	6.5
From my experience	191	78
From neighbors	196	80
Farmers who usually read the labels on pesticide containers	31	12.4
Farmers who could understand the instructions for use	12	4.9
Farmers who used chemicals with instructions in a language they don’t understand	235	95.5
Harmfulness of the chemical pesticides to the environment?		
Not harmful	31	12.7
Moderately harmful	16	6.5
Very harmful	5	2
Do not know	192	78.4
Farmers who have awareness on the possibility to protect environment from the harmful effects of pesticides	5	23.8
Harmfulness of the chemical pesticides to human health?		
Not harmful	30	12.2
Moderately harmful	58	23.7
Very harmful	89	36.3
I do not know	68	27.8
Farmers who have awareness on the possibility to protect against the harmful effects of pesticides on health	98	66.7
Farmers who have understanding short-term impact of pesticides on their health	142	58
Farmers who have understanding long-term impact of pesticides on their health	25	10.2
Farmers who responded that they had training about use of pesticides	10	4.1
Farmers who need of any further instruction and/or training on pesticide use	155	63.3
Farmers who need any further instruction and/or training on Safe handling use	102	41.6
Khat growing farmers who have knowledge on:		
Names of pesticides you use	156	63.7
The adverse health effects of pesticides on humans	46	18.8
All pesticides have not the same degree of health impact	46	15.8
Pesticides can enter the body through respiratory system	167	68.2
Pesticides can enter through dermal contact	70	28.6
Pesticides enter through mouth into the body	169	69
Pesticide residues may be detected in the soil	58	23.7
Pesticide residues may be detected in the fruits and levels of sprayed tress	64	26.1
Fate of pesticide residues can be in air	57	23.3
Fate of pesticide residues can be in ground water	13	5.3
Biological and natural pest control methods as alternatives to pesticides use for pest control	28	11.4
Pesticides are harmful to fishes and pollinating insect like bee	97	39.5
Pesticides residues may be detected in the food products like milk, meat & crops	11	4.5
Pesticides cannot be legally used in Ethiopia could be used in their village	27	11
Particular pesticides are legal to use in Ethiopia while buying pesticides	42	17.1

and their environmental impacts. Majority (78.2%) of the farmers responded that they had no information on the effects of the pesticides sprayed on the plants and the physical environment. However, significant number of the farmers (59.9%) responded that they know the effects of pesticides on human health. They were then asked whether they knew how they protect themselves against the harmful effects of the pesticides they use. Out of the respondents that know the effect of pesticides on human health, 66.7% reacted that they protected themselves from the harmful health effect of the pesticides. On the other hand, from 8.53% of those farmers who know the impact of pesticides on the environment, only 23.8% believed that it is possible to protect the environment from harmful effects caused by pesticide use (Table 2).

Perception of the farmers on short and long term exposures to pesticides was also tested. More than half of the respondents (58%) answered that use of pesticides has short term impact on human health. The remaining 42% respondents expressed that they do not know the implication of short-term effects on health or there is no short term effect due to the use of pesticides on their health. Similarly, it was noted that majority of the participants (89.8%) were noted to have no knowledge regarding the long term impact of pesticides on human health. Only 10.2% of the farmers responded that they know the long term effect of pesticides on human health.

The knowledge level of the participants on the routes of entry of the pesticides into the body was also evaluated. All possible routes of pesticide entry were included as alternatives and then perceptions of the farmers were tested. Accordingly, 68.2% of the farmers responded that pesticides can enter through the respiratory system. Another independent question was also asked to learn whether pesticides enter the body through mouth or not, and 69% of the farmers agreed that mouth is the route of entry while all the remaining farmers responded that they are not aware of the pesticide entry to the body through any one of the system. On the other hand, majority of the farmers (71.4%) denied the entry of the pesticides through the dermal contact.

In order to evaluate the farmers' perception on the fate of pesticide residues in the environments, questionnaires were designed and the farmers were asked to forward their views. It was noted that only 23.7% of the participants agreed that pesticide residues can be detected in the soil while 26.1% answered that pesticides can be detected in the fruits and leaves of the trees. Similarly, fewer proportions of the participants, 23.3%, agreed that pesticide residues can be detected in air. Majority of the participants, 94.7%, however, denied the detection of pesticides in underground water. Furthermore, the farmers' knowledge on the impact of pesticides on bees, useful insects and other living organisms was also assessed. The outcomes indicated that 39.6% of the farmers have some information and thus have awareness on the harmful effects of pesticides on insects such as bees and other pollinating insects. The remaining significant number, comprising 60.4% of the farmers, responded that they do not have the necessary knowledge about the harmful effects of pesticides on these insects. Besides, smaller percentage of the respondents, 4.5%, agreed that pesticides residues could be detected in food samples such as milk, meat and crops.

Participating farmers were also asked about their past experiences of using any other alternative pest control techniques meant for reducing the quantities of pesticides regularly used. Most of the respondents, 88.6%, replied that they had no past experiences of using alternative methodologies apart from the continuous use of chemical pesticides every year. However, 11.4% of the farmers responded that they sometimes use traditional methods such as smoke, detergent *(omo*) and urine of their cattle. It has also been learned, from the overall responses of the farmers, that the concept and practices of integrated pest management were not known and that the concept was also not clear to them.

In another set of questions, perception of the farmers on legality of the pesticides imported was evaluated. It was noted, from their responses, that they know the pesticides importation legality in different ways. Accordingly, 13.1% of the farmers replied that they are aware of the legality of the pesticides they use while 11% of them believed that illegal pesticides were used in their villages. The latter group was further asked, ‘how do they know whether the pesticides used are illegal or not’. Based on their responses, most of them had the perception that pesticides which were provided by the government or unions are considered to be legal, and illegal pesticides could be obtained from illegal markets. The remaining few farmers indicated that agricultural workers are the source of information on the legality of pesticides. However, 39.6% denied the use of illegal pesticides in their villages and 49.4% answered that they do not know whether the pesticides used in their village are illegal or not.

DISCUSSION

Majority of farmers who participated in the data collection were in the age of adult range and had sufficient experiences of *Khat* crop production. The duration of stays of the respondents in the study area is also long enough, and thus the information obtained for the intended study could be reliable. This could be a sufficient condition to conclude that most respondents were permanent residents of the study areas and involved in *Khat* cultivation from their early ages.

Furthermore, as nearly all the study subjects were *Khat* growers, the duration of their stays could linearly be correlated with the number of years they are engaged with *Khat* cultivation. However, the level of education of most of the respondents is low, either no schooling or at the level of basic education.

Poor pesticide handling, application, and storage can negatively impact the health of humans, animals, and ecosystems. One of the primary concerns of this study was to learn whether the farmers know the types and thus the intended use of pesticides they are using. Even though most of the farmers believed that they know the name of pesticides they use, significant percentage of the farmers responded that they were not sure of the name of pesticides they are using. However, it was generally known, from the farmers' opinions during oral discussions, that most of the respondents do not precisely know the names of the pesticide products mainly because of the variety of reasons including less attention given to the names, lack of information and education, the assumption that all pesticides are the same, some products that are repacked and sold in non-original containers that are not labelled, suspicions of the information obtained from the retailers, which are sometimes misleading, etc. As it has also been stressed by the respondents, they were using the type of container in which it is sold to identify the type of pesticide and by their physical state; liquid or solid. The findings of the current study are similar to the reported results of the field study performed on horticulture production areas of Ethiopia (Mequanint et al., 2019; Mormeta, 2019). In another item of this study, knowledge of the farmers on the safe handling and use of pesticides, their health and environmental impacts were also accessed. The compiled results of the study indicated that significant percentage of the farmers were not confident enough in their knowledge of the health and environmental effects of the pesticides used. Similarly, comparable proportions of the respondent farmers have forwarded their views that they have not had the required knowledge and education to protect their health and the physical environment from the hazardous effects of the pesticides they use. However, awareness of *Khat* growing farmers on the health effects of pesticide use and its protection is relatively higher than their awareness on the environmental impact of pesticides and their protection. The differences in awareness may be due to their low level of education to know the effect of pesticides on the environment and its protection. Evaluation of the research findings indicated that little is known about the mechanism of health protection from pesticide hazard. As has also been learned from most of the farmers, during the oral discussion, to prevent the poisonous effects of the pesticide is not to ingest them.

Earlier studies in Pakistan and African countries, including Ethiopia reported that most of the pesticides used by typical farmers are stored inside their house, like kitchen (Mubushar et al., 2019). Similar study conducted in Tanzania, on the other hand, indicated the farmers store pesticides after for next use (Philbert et al., 2019).

On the contrary, findings of the current study reflected that over one third of the respondents described that they do not know the right storing conditions of pesticides and their containers. The basic and safest storing conditions of pesticides such as storing in dry, cool, locked, well ventilated areas; storing at places far from food items; keeping away from fire and storing in tightly closed original containers were used as the major parameters to evaluate the knowledge levels of the farmers using pesticides. Based on the results, it was noted that knowledge of the sampled farmers on the basic storing conditions is limited, although relatively larger proportions of the study participants, 79.4%, have some awareness on particular aspects of storing pesticides; that is, pesticides should be kept out of the reach of children. Additionally, based on the result of the field observations, it was learned that the housing styles of majority of the farmers are single classrooms that are not partitioned and all the activities related to food preparation including cooking, eating, pesticide storing, etc., are all performed there. It is evident that the pesticides stored indoors could increase the degree of exposure of the family members. In particular, children could easily get hold of the pesticides apart from the improperly sealed containers, potentially leaching their contents into the indoor environments which could contaminate their foods.

Educational level has important roles in increasing farmers' knowledge and perception of pesticide risks. Findings of the research works carried out in different countries also revealed that farmers were not receiving regular agricultural extension services and appropriate information on pesticide risks and safety trainings (Cochrane and O'Regan, 2016). In a similar manner, results of the present study indicated that there was no adequate information or training given to the *Khat* growing farmers on the safe use of pesticides. The primary source of information on the use of pesticides is the farmers' traditional experiences which may not be education based. On the other hand, both the agricultural experts and health professionals confirmed that relevant training on awareness creation was never given to the *Khat* growers. It was further realized that no agricultural extension services were given to these farmers, who are *Khat* growers. Recent study report also indicated that there are no planned assistance and encouragements given to the *Khat* growing farmers. Ethiopian government neither encourages nor takes any action against its cultivation, trade and use (Ndayambaje et al., 2019). It looks like there is no specific government policy that promotes or prohibits the production of *Khat*.

Written information on pesticide packaging is one of the information sources on pesticides safe use. In this regard, few farmers responded that they were provided with the necessary information through different means of information sources including labels of the containers. However, unless purposeful training is organized and farmers are given adequate training, presence of the labels on the containers may not be adequate condition for the farmers to extract the required information from the labels. Another factor critically affecting the usability of the direction on the products labels could be the difficulty in reading and understanding of the instructions, as the language used on the labels is different from the local/national languages. In a recent study done in South East Ethiopia, majority of the respondents could not read labels on pesticide containers (Gesesew et al., 2016). On the other hand, the worst scenario recently reported indicated that some farm workers were found reluctant to read manufacturers' labels even if they are capable of reading them (Jallow et al., 2017).

Pesticide exposure in the field occurs mainly through dermal contact and inhalation. The use of personal protective devices is known to greatly reduce the exposure levels before irreparable disease develops (Surgan et al., 2010). The findings of the presented study also revealed that the farmers' knowledge level on the routes of pesticide exposure, that is, oral, dermal or inhalation is highly diversified. Significant proportions of the involved farmers perceived that pesticide can enter through the mouth and respiratory system. However, most of the farmers do not have the knowledge that pesticides can enter the body systems through dermal contacts. Thus, lack of adequate training and the necessary knowledge on the route of entry of pesticides could be the main cause for the risk of exposure to pesticides. As a consequence, the importance of using protective devices during pesticide handling is not well understood along with the end result for entry of pesticides into the body systems (Okoffo et al., 2016).

In a similar manner, lack of awareness and proper management of pesticides may contaminate the atmosphere, water, soil, agricultural products, and consequently result in direct or indirect pollution of the biological systems, food and food products and human health. In this regard, the outcomes of the current study clearly revealed that, in general, level of the farmers' awareness about the fate of pesticide residues in their environment is low. Moreover, almost all the sampled farmers lacked the knowledge of the presence of pesticide residues, after their application, which could contaminate the food items and other household utensils as well as their possible occurrences in the underground waters. However, it was rather a positive outcome that was noted from the subjects regarding the effect of pesticides and their residues on birds, bees and other pollinating insects.

Furthermore, the outcomes of the current study confirmed that majority of the sampled farmers were not aware of the alternative pest management techniques rather than using pesticides. It was however interesting to learn that few of the farmers expressed their traditional experiences of using smoke, detergents and urine of their cattle for controlling pests; though the efficacy and effects on the environment and lives of ecosystems may require further studies. Pesticides banned in the developed world are still entering the developing countries, following several illegal routes that are not clearly known. Use of DDT, for example, was banned in the developing world, though the use was permitted specifically for controlling mosquitoes in the malaria regions of the developing countries, like Ethiopia. Recent survey conducted in Ethiopia also indicated that DDT is still in use as pesticide by farmers for agricultural purposes (Derso and Dagnew, 2019). The survey further described that DDT is openly displayed in the shops for sale. The farmers' perception of the legality of pesticides used, including DDT, was also evaluated in the study. The responses of the sampled farmers reflected that DDT is in use mainly in agriculture than for the control of mosquitoes. Only limited population of the respondents had the awareness that illegal pesticides are used in Ethiopia or in their villages. The findings of the current study are also in good agreement with the study performed in the Rift Valley region, confirming that most farmers of the study areas did not have the awareness that the pesticides they were using are not legally registered; for example, DDT, are used for the purposes that are strictly prohibited by the international standards (Belay et al., 2017).

Future perspectives

The knowledge gaps identified in this study could be used to design knowledge-based interventions focusing on the *Khat* growing farmers. In this regard, integrated efforts from all concerned that may be aimed at the awareness rising of the farmers on proper pesticide management and related issues should be encouraged. Besides this, further studies should be carried out to assess the usefulness and efficiencies of traditionally used methods by some farmers, for example, the smoke, detergents and urine of the cattle. This would require active involvements of various professionals; including health workers, chemical toxicologists, environmentalists, entomologists, chemical analysts among others. As pesticides are continuously applied to the same farmlands, contamination of the environment with their residues may be highly probable. Furthermore, the current study also identified that persistent organochlorine pesticides, such as DDT, are still in use which are expected to persist in the environment for long time and bio accumulate through the food chain particularly in the fatty tissues. As a result, further studies could also be initiated to investigate the extent of contamination of the soil and water bodies by pesticides in the study areas.

In addition to the occupational exposure to pesticides, further exposures could also occur while spraying and storing mainly through the food eaten and drinking water contaminated with the pesticide residues. Non-occupational exposures originating from the pesticide residues in food, air and drinking water generally involve low doses and are chronic. To safeguard the *Khat* growing farmers as well as the general population, who consume the plant food and water from pesticide contaminated areas, further investigations of the level of pesticides in the *Khat* plant and food of both animals and plant origins should be carried out. Consequently, developing effective and reliable analytical methodologies are very important to compare the research findings with the international standards.

Conclusion

In this study, the knowledge and perception of the use of pesticides for pest control on *Khat* production was investigated. The field survey study was carried out around the Haromaya Wereda of the Eastern Oromia Regional State of Ethiopia, on pesticide use of the *Khat* growing farmers. The outcomes of the study indicated that almost all the farmers in the study areas are entirely dependent on *Khat* production both for local consumption and as a means of income through export. Furthermore, it was also learned that the economy of the country is also highly supported by the tax from the *Khat* export. On the other hand, the findings revealed that farmers growing *Khat* are using pesticides, including the banned ones such as DDT, as a means of increasing and improving the production yields. However, the knowledge and perception of the concerned farmers towards the health and environmental risks that could result from pesticide use were found to be limited. Outcomes of the study also revealed that no training and awareness creations were made including the safe handling and uses of the pesticides. Based on the responses from the subjects, the possible entry of the pesticide residues to the body systems, through various routes, was not well known. Besides this, most of the respondent farmers do not read and understand the information provided with the labels of the containers, as these are communicated with the language they are not familiar to and most of them are also illiterate. The overall health risks of the pesticide use thus require special attentions of all concerned bodies.

The aspects requiring future involvements in order to minimize risks of pesticides are also identified and suggested. In addition, the necessities for training and awareness raising programs, the need for investigating usefulness and efficacy of the traditional ways of pest controls used by some farmers were emphasized. Similarly, analysis of the extent of accumulation of pesticide residues and understanding of their chemical compositions/contents are suggested as the measures requiring further collaborative efforts in order to come up with reliable solutions to minimize the probable risks to human health and the inhabitants of the ecosystem.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Short-term response of flat tree oyster, *Isognomon alatus* to CO_2 acidified seawater in laboratory and field experiments

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Seawater changing chemistry has consequences on coastal ecosystems and their living resources. Future projections suggest the pH could drop ~0.2-0.3 pH units by the year 2100 under a business-as-usual (BAU) CO_2 emission scenario. Marine calcifying organisms such as corals, calcifying algae, crustaceans, mussels, oysters and clams are most likely to be impacted by ocean acidification. The *Isognomon alatus* (flat tree oyster) is an important species that can be negatively affected by the lowering of seawater pH. *Isognomon alatus* is an important food source, a substrate for other benthic organisms (e.g., stone crab, *Menippe mercenaria*) and contribute to nutrients recycling in coastal ecosystems. The study was conducted to test the impacts acidified seawater CO_2 on the growth of *I. alatus* under controlled laboratory conditions as well as field experiment. The *Isognomon alatus* lost weight and experienced negative growth rates of –0.56 ± 0.36 mg $g^{-1}day^{-1}$ under average pH values of 7.8 expected by the end of this century compared to a loss of –0.26 ± 0.23 mg $g^{-1}day^{-1}$ under ambient pH (value 8.1) conditions. In contrast, *I. alatus* incubated in a field experiment showed a gain in weight and positive growth of 3.30 ± 0.23 mg $g^{-1}day^{-1}$ despite exposure to pH levels (~7.4) during low tide significantly lower than those experienced in the laboratory. Overall, the results showed concern on the impacts of acidification flat tree oyster (Bivalvia:Isognomonidae). A decline of calcifying bivalves populations can impact coastal ecosystems function and indirectly affect the human beings that depend on them as a food source.

Key words: Ocean acidification, climate change, Isognomonidae, shell dissolution, bivalve's growth, estuarine.

INTRODUCTION

Anthropogenic carbon dioxide (CO_2) emission and the effect of its accumulation in the atmosphere and uptake by the oceans have raised severe concerns for its consequences to Earth's climate and oceanic ecosystems (IPCC, 2007; Andersson et al., 2008; Cole, 2013; IPCC, 2013; Bates et al., 2014). Atmospheric CO_2 has risen mostly due to the burning of fossil fuel from pre-industrial levels of about 280 ppm to current levels of 385 ppm (Caldeira and Wickett, 2005; Doney et al., 2015; IPCC, 2018, 2019). Atmospheric CO_2 concentration is expected to rise to ~750 ppm by the end of this century under a business-as-usual (BAU.) CO_2 emission scenario

(Schmittner et al., 2008; Cole, 2013; IPCC, 2014; Reith et al., 2019). The ocean acts as a natural carbon sink and absorbed about 25 – 30% of anthropogenic CO_2 (Sabine et al., 2004; Byrne et al., 2010; Byrne, 2011; Ryan et al., 2015). Consequently, the global oceanic surface pH of ~8.1 has declined by 0.1 units compared to pre-industrial pH values (~8.2) (Orr et al., 2005; IPCC, 2007, 2019). Numerical models predict a further drop in the ocean pH by 0.2 – 0.5 units by the year 2100 if CO_2 emissions from human activities continue to increase at present rates (IPCC, 2007; Brierley and Kingsford, 2009; Poloczanska et al., 2016).

Atmospheric carbon dioxide gas (CO_2) rapidly equilibrates with the concentration of CO_2 in the surface ocean (Kleypas et al., 2006; Byrne and Przeslawski, 2013; Fabricius et al., 2014; Doney et al., 2020). Therefore, an increase in atmospheric CO_2 raises the average surface seawater partial pressure of carbon dioxide (pCO_2) (Hofmann et al., 2010; Bijma et al., 2013). Increasing CO_2 dissolve in aqueous solution causes the pH to decrease and affects dissolved carbonaceous species in seawater (Doney et al., 2009; Zeebe, 2012; Pettit et al., 2013). Carbon dioxide gas (CO_2) dissolves in seawater to form carbonic acid, dissociating into protons and bicarbonate ions, thereby causing a decrease in seawater pH and carbonate ion concentrations (Orr et al., 2005; Feely et al., 2009; Orr et al., 2015; Baldry et al., 2020). This phenomenon is known as ocean acidification (McNeila and Matear, 2008; Doney et al., 2015; Melendez and Salisbury, 2017). A decrease in carbonate ion concentration (CO_3^{2-}) leads to decreased saturation states (Ω) of calcium carbonate ($CaCO_3$) minerals such as calcite ($\Omega_{calcite}$) and aragonite($\Omega_{aragonite}$), the two common crystalline components of marine organisms (Fabry et al., 2008; Hofmann et al., 2010; Olischläger and Wild, 2020). A reduction in saturation state (Ω) may suppress the rate of calcification, composition, and dissolution of the calcium carbonate ($CaCO_3$) of marine organisms such as foraminifera (Green et al., 1993; de Moel et al., 2009), corals (Langdon and Atkinson, 2005; Andersson and Gledhill, 2013; Fabricius et al., 2014; Kawahata et al., 2019). calcifying marine algae (El Haïkali et al., 2004; Robbins et al., 2009; Costa et al., 2019), coccolithophores (Riebesell et al., 2000; Dissard et al., 2009; Ridgwell et al., 2009; e Ramos et al., 2010), finfish (Ishimatsu et al., 2008; Lacoue-Labarthe et al., 2009), zooplankton (Wang et al., 2018; Campoy et al., 2020), echinoderms (Miles et al., 2007; McClintock et al. 2011; Ross et al., 2011), sea urchin (Miles et al., 2007; Emerson et al., 2017), and shellfish (Bamber, 1987; Bamber, 1990; Shirayama and Thornton, 2005; Bibby et al., 2008; Talmage and Gobler, 2009; Talmage and Gobler, 2010).

There is a great concern about the impact of ocean acidification on marine calcifying organisms (Gazeau et al., 2007; Comeau et al., 2009; Talmage and Gobler, 2009; Ross et al., 2011; Nguyen and Byrne, 2014; Bindoff et al., 2019). Populations of marine animals could respond negatively to the low pH resulting from rising CO_2 (Pörtner, 2008; Widdicombe and Spicer, 2008; Dupont and Thorndyke, 2009; Findlay et al., 2011) and marine invertebrates (Dupont and Thorndyke, 2009; Yu et al., 2011; Watson et al., 2012). Impact of ocean acidification on marine fauna includes reduced growth rates (Michaelidis et al., 2005; Berge et al., 2006; Gazeau et al., 2007), decreased reproductive success (Bibby et al., 2007; Kurihara et al., 2007; Kurihara et al., 2009; Ross et al., 2011; Olischläger and Wild, 2020) and shell dissolution (Bamber, 1990; Green et al., 1993; Shirayama and Thornton, 2005; Findlay et al., 2011). Another effect of seawater CO_2 acidification includes decreased metabolism (Michaelidis et al., 2005; Talmage and Gobler, 2010; Dissanayake, 2014; Liu et al., 2020), acidification of internal body fluids (Spicer et al., 2007), induced defenses (Bibby et al., 2007), increased susceptibility to infection (Holman et al. 2009; Mukherjee et al. 2013), shell thinning (de Moel et al., 2009), impairment of shell formation (Zhang et al., 2020), and impairment of immune function (Bibby et al., 2008; Mukherjee et al., 2013).

A few studies show marine organisms' short-term exposure to ocean acidification (Fabry et al., 2008; Dissard et al., 2009; Fabry et al., 2008; Talmage and Gobler, 2009). The blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) calcification rates are estimated to decrease by 25 and10 %, respectively, following the end of the century, IPCC IS92a B.A.U.Scenario (~740 ppm in 2100) (Gazeau et al., 2007; IPCC, 2007, 2018). Ocean acidification impacts larval development of marine animals such as invertebrates (Kurihara and Shirayama, 2004; Dupont and Thorndyke, 2009), sea urchin (Kurihara and Shirayama, 2004; Martin et al., 2011), fish (Ishimatsu et al., 2008), sea snail (Onitsuka et al., 2014), amphipods (Egilsdottir et al., 2009), decapoda (Dissanayake and Ishimatsu, 2011; Curry, 2020), and gastropods (Bibby et al., 2007; Tagliarolo et al., 2013).

The increased pCO_2 (1500 – 2100 ppm, pH ~ 7.4) of seawater projected to occur by the year 2300 will severely impact the early development of *Crassostrea gigas* (Kurihara et al., 2007; Havenhand and Schlegel, 2009). There was no significant effect on the fertilization success of *Crassostrea gigas* and *Mytilus galloprovincialis* exposed to 2000 ppm CO_2 (~ pH 7.4) treatments

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(Kurihara et al., 2009). There was also no significant effect on the sperm swimming speed, sperm motility, and fertilization kinetics in a population of *Crassostrea gigas* under future ocean acidification levels (−0.35 pH unit change) (Havenhand and Schlegel, 2009).

Isognomon alatus (Gmelin, 1791) (Bivalvia: Isognomonidae), the flat tree oyster, is a sessile intertidal species that attach to hard substrata (Patrick, 1988; Saed et al., 2001; Wilk and Bieler, 2009). This species occurs from North America, Central Florida to Bermuda, the Bahamas, West Indies, Caribbean Central America, the northern coast of South America, and south Brazil (Thomas and Dangeubun, 1994; Abbott and Morris, 1995; Mikelsen and Bieler, 2008; Suarez-Ulloa et al., 2019). It grows on Red mangrove roots (e.g., *Rhizophora mangle*), tidal creek, rocks, and human-made structures (Siung, 1980; Patrick, 1988; Leal et al., 2019).

The study assessed a short time culture of *I*. *alatus* in manipulated seawater CO_2 concentrations (193 ppm, 390 ppm, and 766 ppm) and field experiment. Coastal ecosystems are most vulnerable to climate change stressors such as ocean acidification. The study's chief objective was to evaluate the impact of ocean acidification on the culture of *I*. *alatus* in 45 days experiment. The research seeks to understand the effect of lowering pH (~ 7.8 – 7.9) due to the manipulated levels of CO_2 concentration in seawater on the growth of *I*. *alatus* cultured in laboratory and field experiment with natural pH gradient (8.1 – 7.9) in Mangrove Bay Estuary, Bermuda. Knowledge of the impacts of ocean acidification on marine animals such *I*. *alatus* is critically important to understand the lowering of ocean pH and its consequences on coastal ecosystems. The short-term response of flat tree oyster is useful for predicting the impacts of climate change stressors such as seawater lowering pH on the bivalve's population to improve our understanding of their survival, mortality, and adaptations. The findings provide chemical, ecological knowledge about the flat tree oyster response to lowering pH to improve management of bivalves in mangrove system.

MATERIALS AND METHODS

Collection of *I. alatus*

Eighty-four specimens of the flat tree oyster, *Isognomon alatus* were randomly collected from attached rocks and mangroves at low tide in Mullet Bay Estuary (32° 22' 30''N, 64° 41' 35''W), St. George, Bermuda on 21st January 2009. Mullet Bay Estuary (an intertidal mudflat) is situated at the Northwestern extension of St Georges Harbour, south of St. Georges Island (Mackenzie et al., 1970; Zablocki et al., 2011). The species (Figure 1a) was first allowed to acclimatize (Figure 1b) to Naess laboratory conditions at the Bermuda Institute of Ocean Science (BIOS). The specimens were placed in a labeled Petri dish. They were then transferred into experimental tanks connected to a continuous seawater flow-through system with its source from Ferry Reach, North Atlantic Ocean, Sargasso Sea, Bermuda. The laboratory and field experiments were conducted from January 2009 to April 2009.

Laboratory experiment

Manipulation of CO_2 acidified seawater for the culture of I. alatus

A seawater flow through the system provided continuous flowing natural seawater into two head tanks (control and altered CO_2) placed at an elevated location above the experimental tanks (Figure 2). Tygon tubes (I.9 m) connected experimental tanks (n = 6) through head tanks, which then supplied continuous seawater at the same flow rate of 60 ml/m with gravitational force into individual tanks. Both head tanks initially had natural seawater (pH 8.1 – 8.2) flowing through them. Pure (100 %) carbon dioxide gas (CO_2) was bubbled into the natural seawater to alter the chemistry in one head tank. Three randomly selected control tanks (control, C1, C2, and C3; n = 3) were held under ambient seawater conditions, and other three tanks, the pH was altered by CO_2 bubbling. The altered pH in acidification treatment tanks (T1, T2, and T3, n = 3) was achieved by adjusting partial pressure of carbon dioxide (pCO_2) using various CO_2 air mixtures (T1 = 193 ppm, T2 = 390 ppm, and T3 = 766 ppm). CO_2 is measured in parts per million (ppm), while pCO_2 is measured parts per million per volume (ppmv). The concentration of CO_2 was measured with Carbon Coulometer. Flow rates of air and concentrated CO_2 gas were controlled at 12.0 ml/min from time to time using adjustable Agilent Flowmeter ADM1000.

Seawater samples were collected from the experimental tanks by filling 200 ml Kimax glass bottles to determine dissolved inorganic carbon (DIC) and total alkalinity (TA). The glass bottles were taped with Teflon tape before sampling to prevent atmospheric equilibration and provide good closure. Seawater samples were preserved with 0.1 ml of saturated mercuric chloride solution ($HgCl_2$) and stored at 4°C until analysis (Huang et al., 2012).

Total alkalinity is quantified with a Gran titration (Gran, 1952) but has been modified for an open-cell titration method quantified with nonlinear least-squares calculation (Dickson, 1981; Bradshaw and Brewer, 1988; Dickson et al., 2003). Dissolved inorganic carbon (DIC) is commonly measured by acidifying a known volume of water to release CO_2, which is then absorbed into an organic base solution and titrated with a coulometric detector (Johnson et al., 1987; Johnson et al., 1993; Cole, 2013) or which is quantified with an infrared detector (Goyet and Snover, 1993; Dickson and Goyet, 1994; DOE, 1994). Dissolved inorganic carbon (DIC) commonly consists of CO_3^2, HCO_3, and CO_2 (aq) (Andersson et al., 2008; Yan et al., 2020), is a principal constituent of seawater, and a good tracer of ocean acidification (Bates et al., 2014; Yan et al., 2020).

Total alkalinity (TA) was determined using an open-cell potentiometric acid titration with a precision of 0.04% (~0.8 µmoles kg^{-1}) (Dickson, 1981; Keeling, 1993; DOE, 1994; Bates et al., 1996). Dissolve inorganic carbon (DIC) was analyzed using an infrared AMICA DIC analyzer employing a Li-Cor 6262 NDIR analyzer with a precision of ~0.07% (~1.5 µmoles kg^{-1}) (http://www.bios.edu/Labs/co2lab/research/CO2_instrumentation.html) (Bates et al., 2012). Two junk samples and certified reference materials (CRMs) were run before the measurement of dissolved inorganic carbon and total alkalinity. Certified reference materials (CRMs) have been developed to control the quality of TA and DIC analysis (Dickson et al., 2003, 2007). The dissolved inorganic carbon (DIC) and total alkalinity (TA) were measured relative to certified reference material (CRM) (Dickson et al., 2007). The certified reference materials (CRMs) used in this study for DIC was 2011.39 µmoles kg^{-1} while TA was 2183.10 µmoles kg^{-1}. The DIC analysis accuracy was assessed with the difference between the measured CRM value and the certified CRM value (Huang et al., 2012). Correction is done only for dissolved inorganic carbon readings. The correction factor is obtained as CRM actual minus CRM input readings. The corrected value (thus CRM actual minus CRM input readings) was added to the value obtained for the

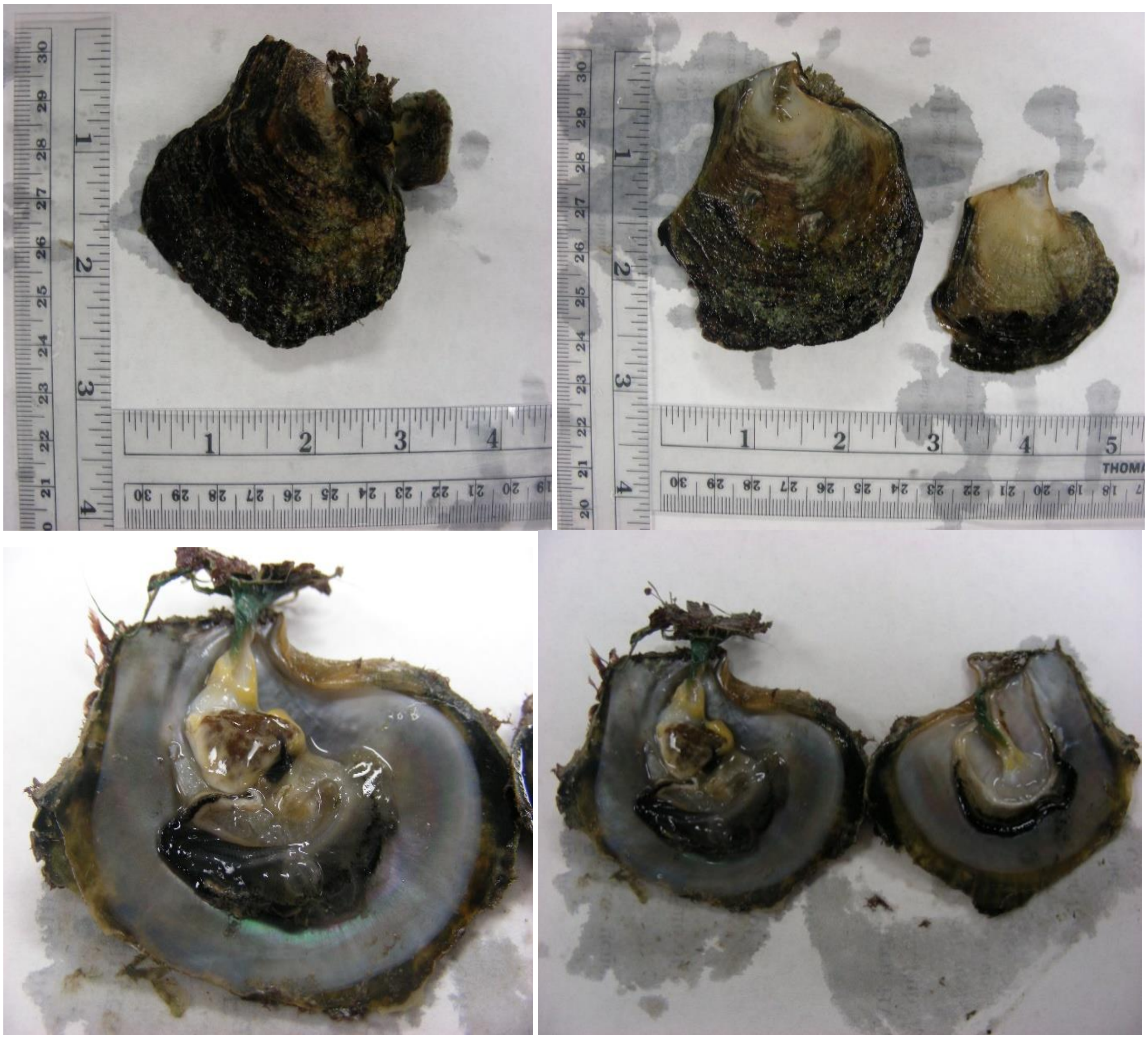


Figure 1. (a) Flat tree oyster, *Isognomon alatus* (Gmelin,1791).

Figure 1. (b) Acclimatization of *I. alatus* to Naess laboratory conditions at Bermuda Institute of Ocean Sciences, Bermuda.

Figure 2. A set up of manipulated CO_2 acidified seawater flow-through system at Bermuda Institute of Ocean Science. The experimental tanks (control tanks, n = 3 and acidification tanks, n = 3. Physicochemical parameters in the experimental tanks were measured using YSI 556 Multiple Parameter Handheld Sonde.

dissolved inorganic carbon (DIC) readings recorded by AMICA system to get the final DIC values.

Carbonate parameters such as partial pressure of carbon dioxide (pCO_2), dissolved carbon dioxide [CO_2], bicarbonate [HCO_3^-] and carbonate [CO_3^{2-}] concentrations, pH_{tot} (total H+ scale) were calculated at *in situ* temperature and salinity conditions based on total alkalinity (TA) and dissolved inorganic carbon (DIC) data (Jones et al., 2016) and then computed by adopting the first and second dissociation constants of carbonic acid (Mehrbach et al., 1973; Dickson and Millero, 1987) and calcite ($\Omega_{calcite}$) and aragonite ($\Omega_{aragonite}$) saturation using stoichiometric solubility products for the respective crystalline forms using CO2SYS software (Dickson and Millero, 1987; Orr et al., 2015).

Control tanks were under ambient seawater conditions with usual seawater carbonate chemistry (Table 1). Carbon dioxide gas (CO_2) was bubbled into one head tank to adjust pH; the initial adjusted pH ranged from 7.63 to 7.76. Each acidification treatment tank (T1 – T3) was connected to an appropriate carbon dioxide (CO_2) gas to alter carbonate chemistry (Table 1). However, seawater from Ferry Reach influence daily variation in water chemistry in the experimental tanks (Table 1). The daily changes in pH between the control tanks were pH = 8.1 – 8.2. While in the acidification tanks, adjustable pH was 7.8 – 7.9, about ~0.3 – 0.4 pH units, similar to the expected drop in ocean pH predictions by IPCC BAU IS92a scenario in the year 2100 (Caldeira and Wickett, 2003; Caldeira and Wickett 2005; IPCC, 2007; Findlay et al., 2011; IPCC, 2019). Calibrated YSI 556 Multiple Parameter Handheld Sonde (www.ysi.com) was used to measure physicochemical variables such as temperature, salinity dissolved oxygen concentration and saturation, and pH in the experimental tanks (Figure 2). In the experimental setup, physicochemicals were monitored for several days to ensure stable environmental conditions before transferring *I. alatus* into the tanks. Forty-two specimens of *I.alatus* were transferred and distributed randomly among control tanks (C1, C2, and C3) and acidification tanks (T1, T2, and T3). Each tank contains seven of *I.alatus* individually placed in labeled petri-dish. *I. alatus s*hell dimensions (height, length, thickness, and buoyant weight) were determined at the start of the culture and biweekly to monitor shell growth dynamics.

Field experiment

I. alatus was cultured in Mangrove Bay Estuary (32°37'16''N, 64°41'38''W) (Figure 3a). The estuary is situated approximately 100 m east of the Bermuda Institute of Ocean Sciences (BIOS) on the northern shore of Ferry Reach, St. George's Parish, Bermuda

Table 1. Seawater chemistry of experimental tanks in the laboratory set up.

Set-up tanks	Carbonate chemistry				Ocean acidification indicators			Physicochemical	
	DIC (µmoles kg^{-1})	TA (µmoles kg^{-1})	pCO_2 (ppmv)	CO_2 (ppm)	pH	Calcite ($\Omega_{calcite}$)	Aragonite ($\Omega_{aragonite}$)	Temp (°C)	Sal [PSU]
Control	2087.59	2380.77	357.2	Ambient	8.09	3.18	4.90	18.71	36.73
Acidification									
T1	2065.07	2395.42	306.4	193	8.15	3.55	5.47	18.80	36.84
T2	2087.07	2382.33	358.2	390	8.09	2.69	3.19	18.79	36.87
T3	2140.61	2382.78	464.5	766	7.99	2.69	4.24	18.84	36.85

CO_2 is measured in parts per million (ppm) while pCO_2 is measured parts per million per volume (ppmv). Acidification tanks (T1 – T3); DIC = Dissolved inorganic carbon, TA = total alkalinity; pCO_2 = Partial pressure of carbon dioxide; CO_2 = carbon dioxide. Saturation states of calcite = $\Omega_{calcite}$ and aragonite = $\Omega_{aragonite}$, physicochemical (Temp = temperature; Sal = salinity). PSU = Practical Salinity Unit. Manipulated CO_2 (ppm) = 193 (pre industrial), 358. 2 (current) and 766 (future projected level).

(Mackenzie, 1976). Mangrove Bay Estuary covers an area of 3,350 m^2, an average depth of 1.014017 m with thick strands of red (*Rhizophora mangle*) and black mangroves (*Avicennia nitida*) (Mackenzie, 1976) (Figure 3b). Three stations were randomly selected due to a natural gradient in CO_2 and pH along that transect in Mangrove Bay Estuary (Figure 3b) (Mackenzie, 1976). The stations are station A, inside the bay (32°22'16''N, 64°41'38''W), station B, middle of the bay (32°22'16''N, 64°41'39''W), and station C, the mouth of the bay (32°22'17''N, 64°41'40''W) (Figure 3b–c). Shell dimensions of 42 oysters were measured before placement in the stations (A, B, and C). Each tank contained oysters(n = 7 oysters/ tank). Each station (Figure 3c) is replicated (A1, A2, B1, B2, C1, and C2).

Physicochemical conditions and growth of *I. alatus*

The state of physicochemical in-laboratory and field culture characteristics such as seawater temperature (±0.1°C), salinity (±0.01 PSU), dissolved oxygen saturation and concentration (±0.01 mg L^{-1}), and pH (±0.01 units) were measured using calibrated YSI 556 Multiple Parameter Sonde.

Shell dimensions (height, length, thickness, buoyant weight, and growth) of *I. alatus* were measured before the experiment, at 2–3 weeks intervals, and at the end of the experiments. Shell height (maximum distance between the dorsal hinge and the ventral shell margins), shell length (maximum anterior-posterior distance) (Figure 4a), and thickness of *I. alatus* were measured to the nearest 0.1 mm using a Vernier caliper (Figure 4b).

Growth of *I. alatus* was calculated as either increase or decrease in buoyant weight using the buoyant weighing technique (Figure 4c).*Isognomon alatus* weight was obtained by weighing in a constant seawater temperature (21°C) throughout the experiment using an Adventurer Pro AV53 electronic balance scale (± 0.001 g) placed over a seawater bath. Flat tree oysters were suspended in the water bath from a hook underneath the electronic weighing scale (Figure 4c). A reference weight (stainless steel nut) was used by weighing before each measurement in the air (thus on the pan loader) and seawater to achieve constant air weight and buoyant weight. Growth rate (G) is defined as changes in the total weight of oysters. Growth rate (G) is expressed in per gram oyster per day (mg $g^{-1}day^{-1}$) and calculated using the formula:

$$G = [M_{t+1}-M_t]/ [M_t\times(T_{t+1}- T_t)] \quad (1)$$

Where, M_t and M_{t+1} are the oysters weight (g) at the beginning (T_t) and the end (T_{t+1}) of each growth interval.

Statistics

Growth of *I. alatus* in the laboratory (thus controls and acidified tanks) and field culture were subjected to student t-test and one-way analysis of variance (ANOVA), respectively using the Statistical Package for Social Sciences (SPSS) version 12.0 (Kinnear and Gray, 1999). The strength of the relationship between two shell morphometrics parameters established via power regression analysis using Excel Spread Sheet (Yadav, 2018).

Data archive

The research data are stored at the PANGAEA repository. The DOI: 10.1594/PANGAEA.921678.

RESULTS

Physicochemical conditions

Laboratory experiment

In experimental tanks, physicochemical conditions were not constant but fluctuated over the study period (Figure 5a–d). The average temperature progressively increased in the experimental tanks throughout the duration of the study, with occasional drops in temperature associated with

Figure 3. (a). A map of Mangrove Bay Estuary, Bermuda.

cold fronts moving over Bermuda. The seawater temperature in the tanks ranged from 16.5 to 20.7°C, with no significant differences between control and acidification tanks (Figure 5a). Salinity ranged from 36.3 to 36.8 (PS.U.) in control and acidification tanks (Figure 5b). Unexpectedly, from 11 to 18th March 2009, average salinity was slightly higher in acidification tanks than in control tanks due to reduced seawater flow rate and a higher evaporation rate because Tygon tubes that supplied seawater were partially blocked with organic particles. After cleaning the tubes, there was no observed difference in salinity in experimental tanks.

Dissolved oxygen concentration ranged from 4.8 to 6.5 mg L^{-1} in the control tanks and 4.8 to 6.6 mg L^{-1} in the acidification tanks (Figure 5c). Dissolved oxygen saturation (%) did not differ among the experimental tank and ranged from 63.4 to 88.8% (Figure 5d). Seawater pH ranged from 8.0 to 8.3 in the control tanks and 7.7 to 8.0 in the acidification tanks (Figure 5d). The control and acidification tanks maintained a relatively constant separation in pH levels (cultured at different levels of CO_2) throughout the experiment. There is no variation in the measured physicochemical variables in the experimental tanks such as temperature, salinity, dissolved oxygen, except pH (Figure 5a–c; Table 2). Average pH values (± standard deviation) in control tanks (8.10 ± 0.10) varied significantly ($p < 0.05$) from pH in acidification treatment tanks (7.80 ± 0.10) (Figure 5d;

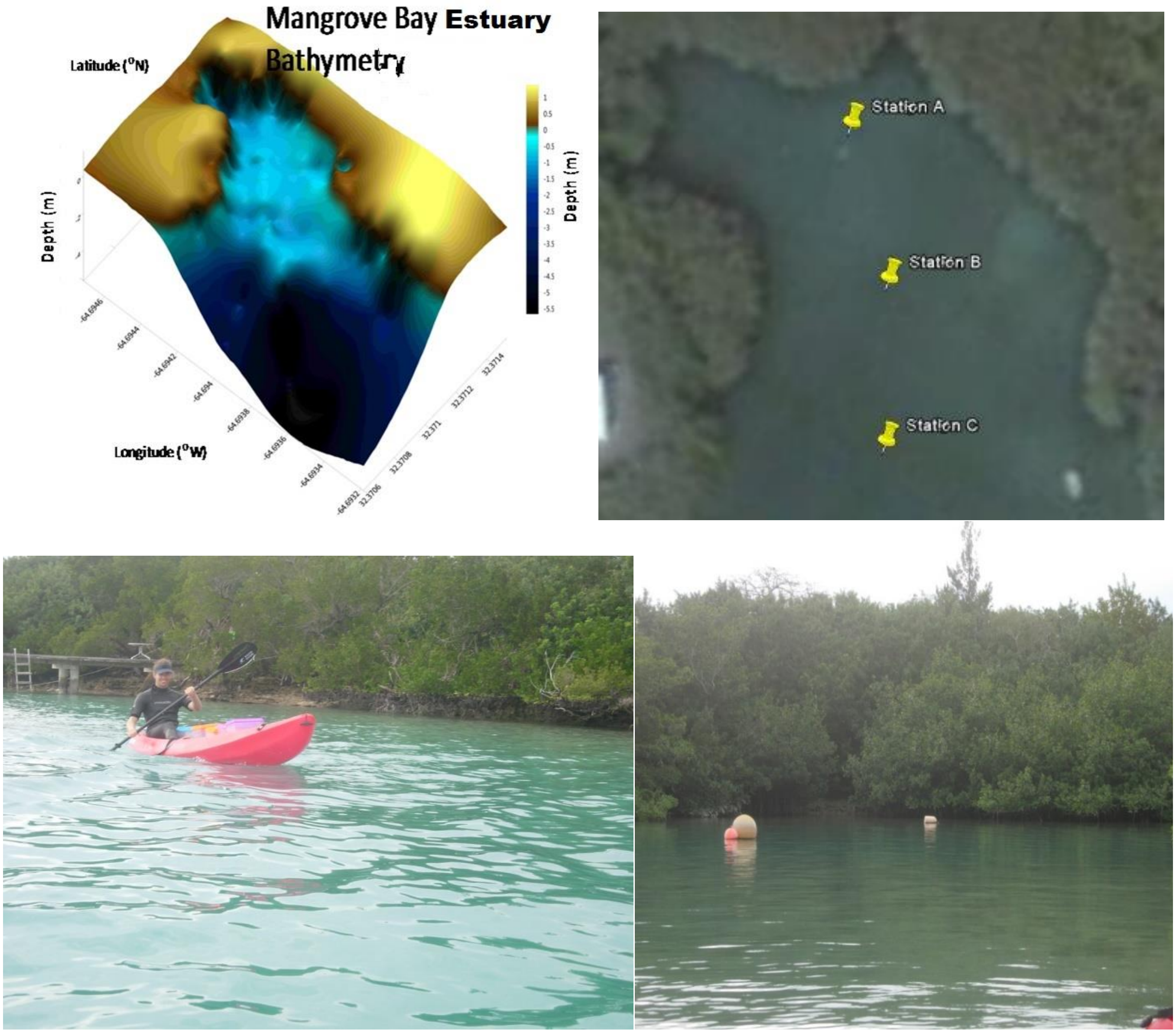


Figure 3. (b) Bathymetry map and the location of three-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Table 2).

Field experiment

Physicochemical conditions in Mangrove Bay Estuary are strongly related to tidal regime changes (Figure 6a–c; Table 3). The minimum temperature (16.74°C) and high salinity (36.8 PSU) were recorded at high tide. The maximum temperature (21.24°C) and low salinity (34.9 PSU) were recorded at low tide (Figure 6a–b). Dissolved oxygen concentrations varied from 5.3 to 7.9 mg L^{-1} (Figure 6c). The minimum dissolved oxygen concentration was recorded at low tide (Figure 6c). Ph ranged from 7.76 to 8.28; the minimum values were recorded at low tide and maximum pH was recorded at high tide (Figure 6d). There was no significant ($p > 0.05$, Table 3) variation in the measured physicochemical parameters among the stations (A, B, and C) in the Mangrove Bay Estuary, Bermuda.

Determination of shell dimensions and growth of *I. alatus*

Laboratory and field experiments

Isognomon alatus shell dimensions (height, length and

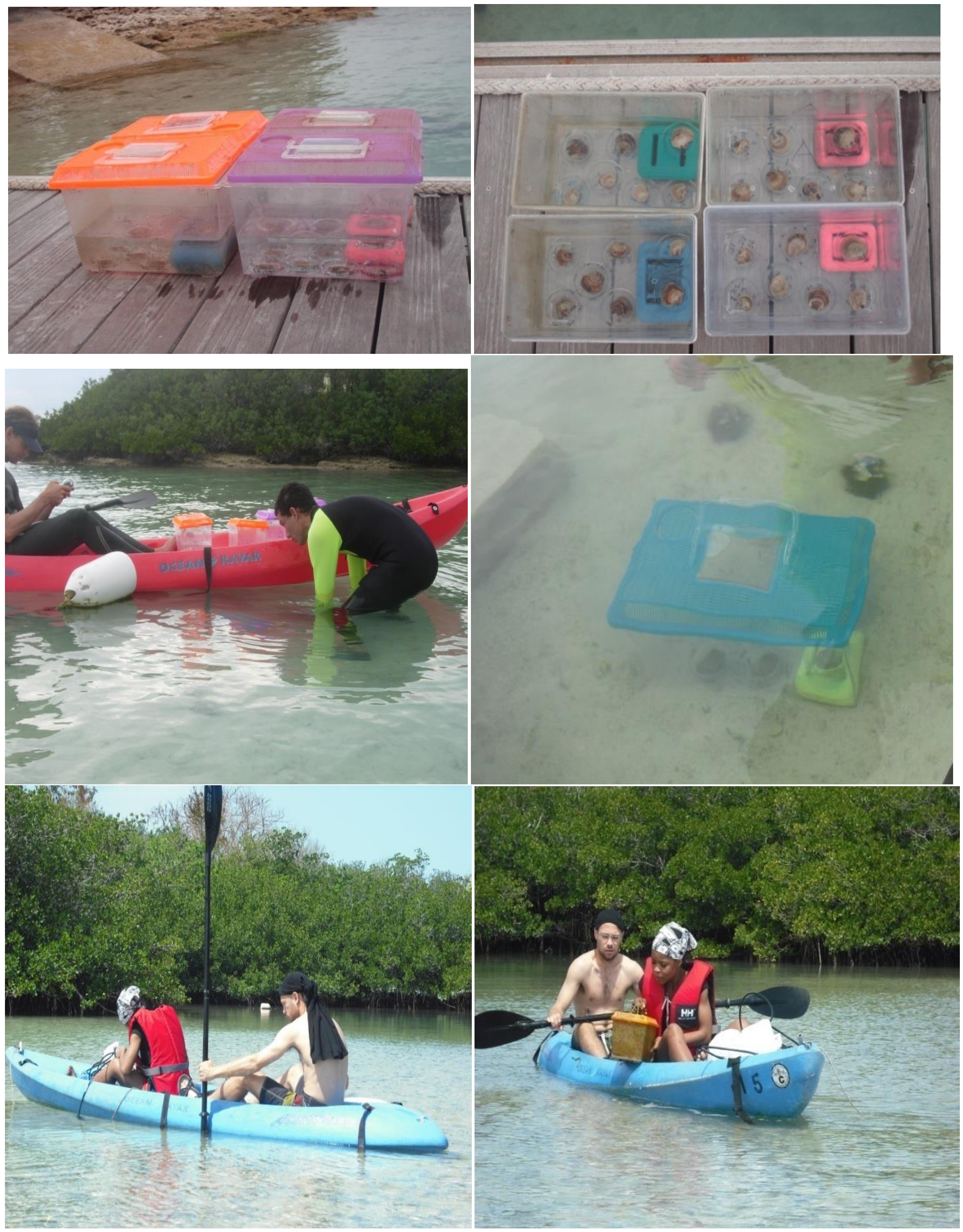


Figure 3. (c) The field culture of *I*. *alatus* at Mangrove Bay Estuary, Bermuda. Photos illustrate placement and retrieval of tanks from the estuary to determine shell dimensions of *I*. *alatus*.

buoyant weight) (Tables 4 and 5) differ in the laboratory and field experiments. The power regression analysis established an association (correlation of coefficient, R^2) between two measured shell dimension parameters (Tables 6 and 7). There was no change in thickness of *I*. *alatus* placed in the experimental tanks (Table 4 and 5). There was no change in the shell height, and shell length of *I*. *alatus* placed in the control tanks. In contrast, there is variation in shell height and length of *I*. *alatus* cultured in acidification treatment tanks (Figure 7a, b). There was a decrease in buoyant weight and negative growth of *I*. *alatus* in both control tanks and acidification treatment tanks (Figure 7c). The growth rate of *I*. *alatus* in the control and acidification tanks remained relatively constant over the entire growth period (Table 8).

However, there was a significant ($p < 0.05$) decreased

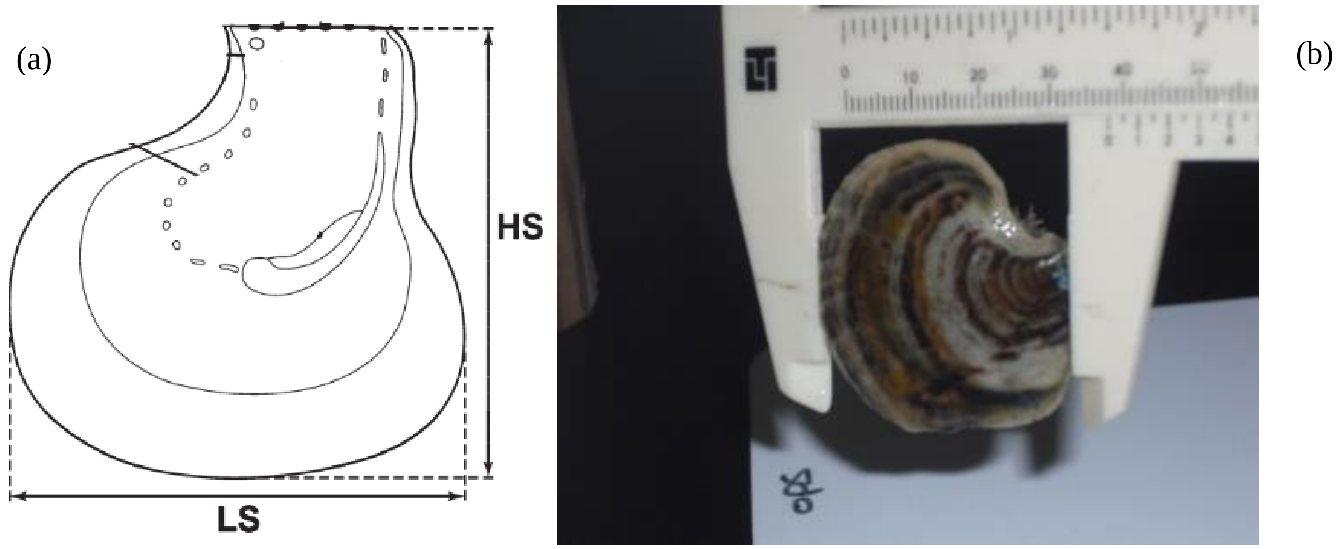
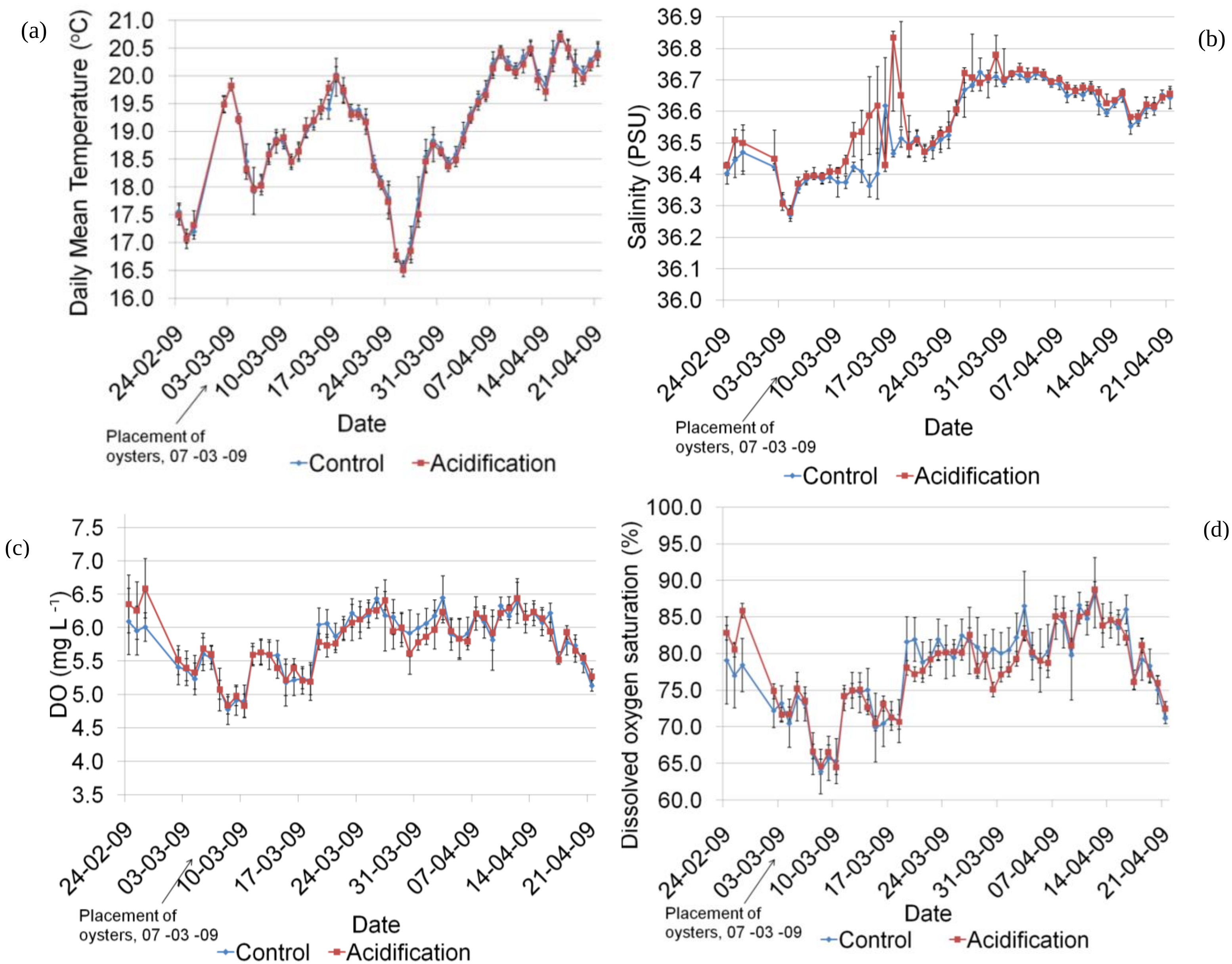


Figure 4. (a) Sketch of bivalve shell dimensions (thus shell length = LS and shell height= HS). (**b**) Determination of shell dimensions (height and length) of *Isognomon alatu*s using Vernier caliper.

Figure 4. (c) Determination of shell weight of *Isognomon alatus* by buoyant weighing technique.

weight and negative growth of *I*. *alatus* cultured at the present pH (8.1 - 8.2) compared to those cultured at future low pH (7.8 - 7.9) (Table 8). In the first growth period, *I*. *alatus* in the control tank weight loss at a growth rate of -0.27 ± 0.03 mg $g^{-1}day^{-1}$ while those in acidification treatment tanks reduced weight at a growth rate -0.55 ± 0.40 mg $g^{-1}day^{-1}$. In the second growth period (24 days of exposure), *I*. *alatus* in the control tanks decreased in the growth rate of -0.25 ± 0.32 mg $g^{-1}day^{-1}$, whereas those in acidification tanks decreased -0.57 ± 0.59 mg $g^{-1}day^{-1}$. Overall, *I*. *alatus* cultured in control tanks reduced weight at the growth rate of -0.26 ± 0.23 mg $g^{-1}day^{-1}$ and those cultures in acidification tanks reduced the growth rate of -0.56 ± 0.36 mg $g^{-1}day^{-1}$ throughout the entire study period of 45 days (Figure 7a–c; Table 8).

There was no clear trend of changes in shell dimensions (height and length) (Figure 8a–b) and growth (Figure 8c) of *I. alatus* along the 3-station transect in Mangrove Bay Estuary, Bermuda. The initial growth of *I. alatus* determined after three weeks of placement at Mangrove Bay was higher than in the rest of the study period (Figure 8a–c). There was positive growth exclusive of some cases (growth period 3) in station C (Table 9). *I. alatus* cultured was slow in the field experiment but much slower at station C (Figure 8c).


(a)
Daily Mean Temperature (°C)
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(b)
Salinity (PSU)
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(c)
DO (mg L -1)
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(d)
Dissolved oxygen saturation (%)
Date
Placement of oysters, 07 -03 -09
Control
Acidification

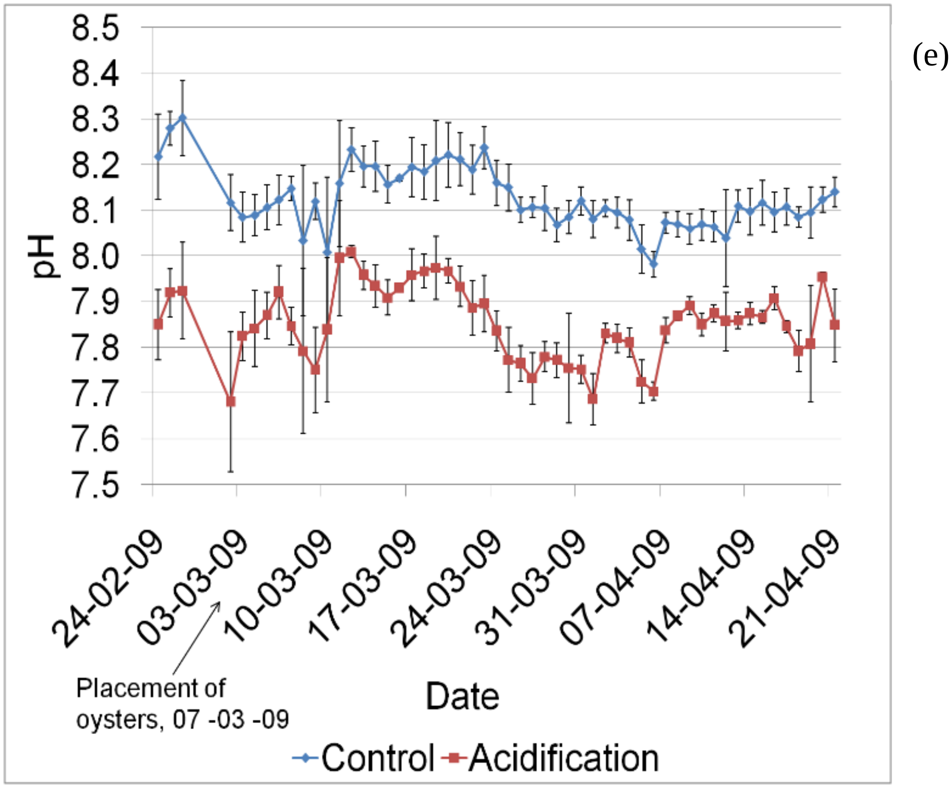


Figure 5. Average mean of daily physiochemical parameters: (a) temperature (°C), (b) salinity, (c) dissolved oxygen concentration (mg L^{-1}), dissolved oxygen saturation (%) (d), and (e) pH recorded in the experimental tanks. Error bars indicate the daily standard deviation from the mean of each parameter.

Table 2. Arithmetic mean (± **S.D.)** of physicochemical conditions in control and acidification tanks.

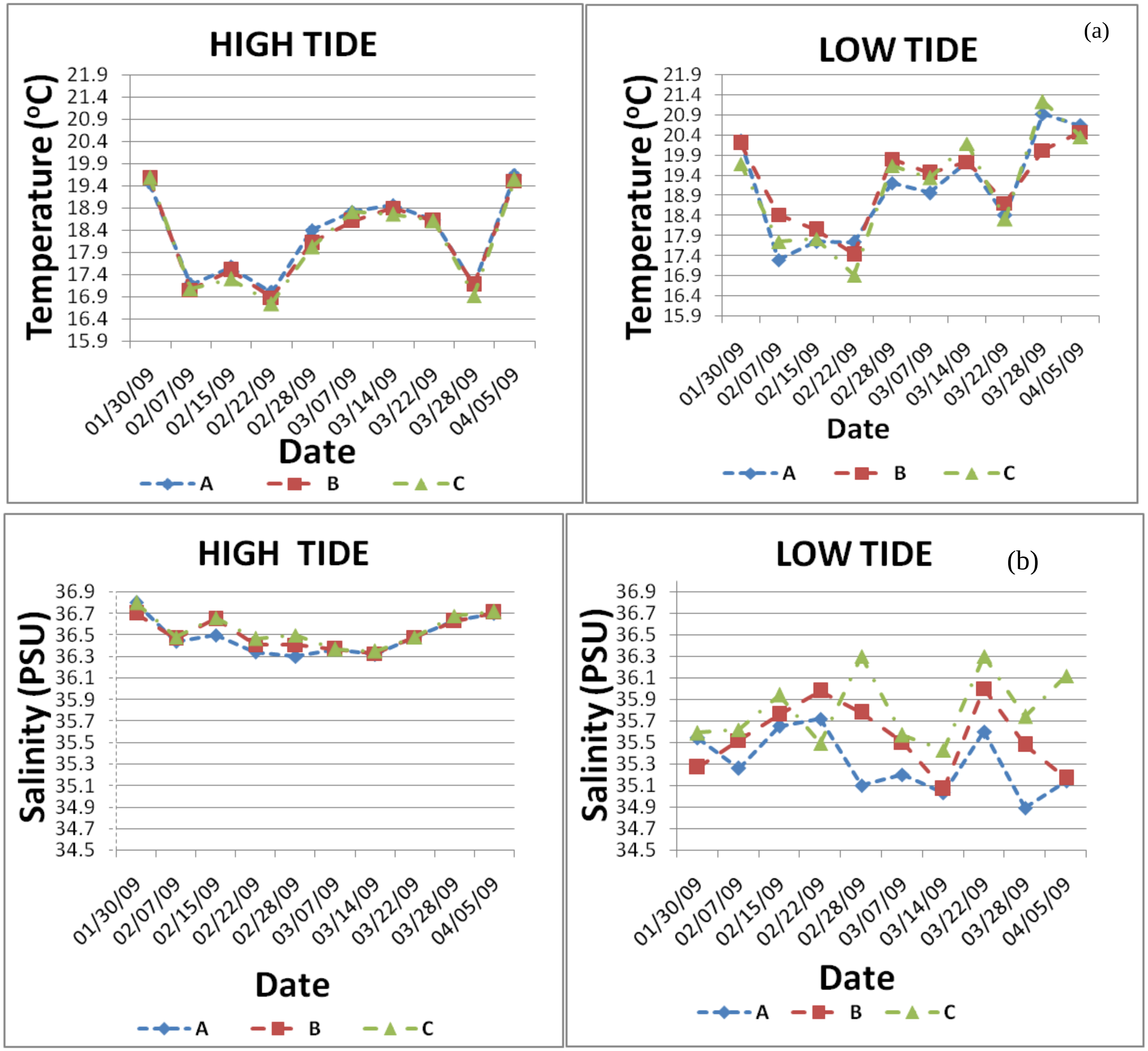
Physicochemical parameters (units)	Control	Acidification	F-value	P-value
Temperature [^{o}C]	19.39 ± 3.98	19.03 ± 1.09	0.88	> 0.05
Salinity [PSU]	36.59 ± 0.98	36.64 ± 1.12	0.68	> 0.05
DO [mgL^{-1}]	6.10 ± 1.89	7.30 ±1.90	0.96	> 0.05
DO [%]	79.04 ± 14.87	79.16 ± 21.30	0.54	> 0.05
pH	8.10 ± 0.10	7.80 ± 0.10	115.02	< 0.05

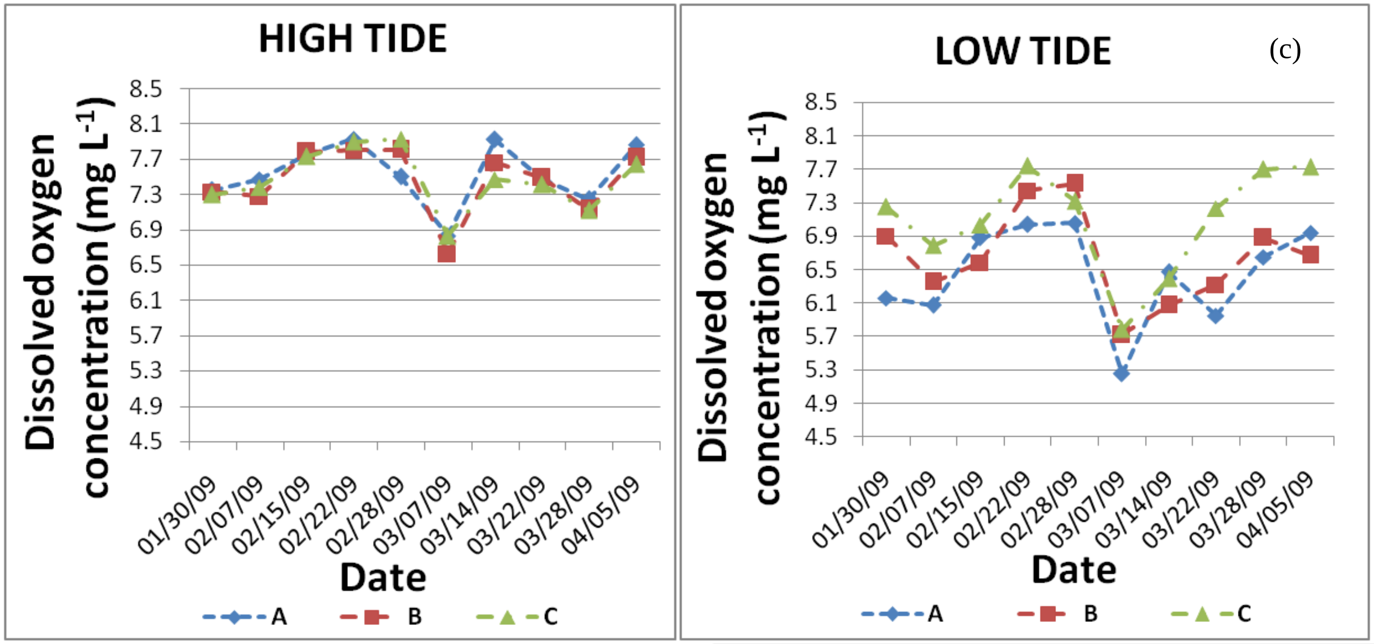
Mean ± S.D.; S.D. = Standard deviation, PSU = Practical Salinity Unit.

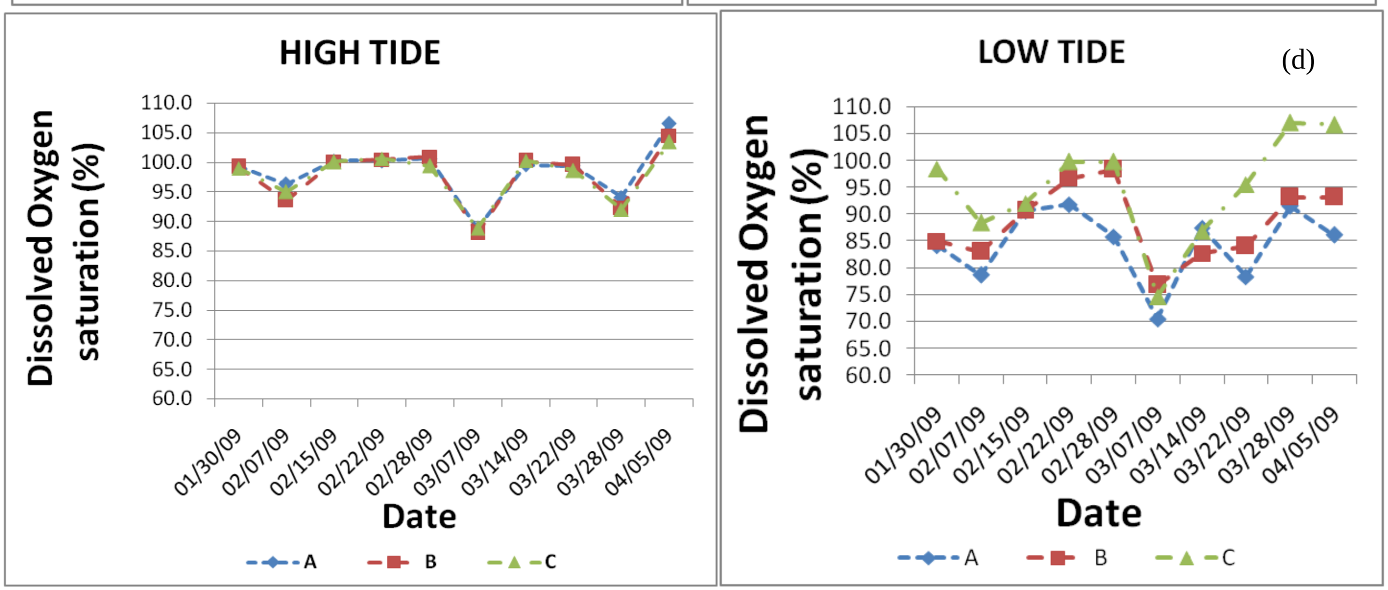
Table 3. Physicochemical parameters measured at Mangrove Bay Estuary, Bermuda.

Parameters (units)	Minimum	Maximum	Mean ± S.D.	F-value	P-value
Temperature [^{o}C]	16.74	21.24	18.65 ± 1.26	1.31	> 0.05
Salinity [PSU]	34.90	36.80	36.09 ± 0.55	3.94	> 0.05
DO [mg L^{-1}]	5.30	7.90	7.2 ± 0.51	0.76	> 0.05
DO [%]	70.50	107.0	94. 96 ± 7.18	1.46	> 0. 05
pH	7.76	8.28	8.28 ± 0.13	1.42	> 0.05

S.D. = Standard deviation; PSU = Practical Salinity Unit.


HIGH TIDE
Temperature (ºC)
21.9 21.4 20.9 20.4 19.9 19.4 18.9 18.4 17.9 17.4 16.9 16.4 15.9
01/30/09 02/07/09 02/15/09 02/22/09 02/28/09 03/07/09 03/14/09 03/22/09 03/28/09 04/05/09
Date
A B C
LOW TIDE
(a)
Temperature (ºC)
21.9 21.4 20.9 20.4 19.9 19.4 18.9 18.4 17.9 17.4 16.9 16.4 15.9
01/30/09 02/07/09 02/15/09 02/22/09 02/28/09 03/07/09 03/14/09 03/22/09 03/28/09 04/05/09
Date
A B C
HIGH TIDE
Salinity (PSU)
36.9 36.7 36.5 36.3 36.1 35.9 35.7 35.5 35.3 35.1 34.9 34.7 34.5
01/30/09 02/07/09 02/15/09 02/22/09 02/28/09 03/07/09 03/14/09 03/22/09 03/28/09 04/05/09
Date
A B C
LOW TIDE
(b)
Salinity (PSU)
36.9 36.7 36.5 36.3 36.1 35.9 35.7 35.5 35.3 35.1 34.9 34.7 34.5
01/30/09 02/07/09 02/15/09 02/22/09 02/28/09 03/07/09 03/14/09 03/22/09 03/28/09 04/05/09
Date
A B C


HIGH TIDE
Dissolved oxygen concentration (mg L-1)
8.5
8.1
7.7
7.3
6.9
6.5
6.1
5.7
5.3
4.9
4.5
01/30/09
02/07/09
02/15/09
02/22/09
02/28/09
03/07/09
03/14/09
03/22/09
03/28/09
04/05/09
Date
A
B
C
LOW TIDE
(c)
Dissolved oxygen concentration (mg L-1)
Date
A
B
C


HIGH TIDE
Dissolved Oxygen saturation (%)
110.0
105.0
100.0
95.0
90.0
85.0
80.0
75.0
70.0
65.0
60.0
01/30/09
02/07/09
02/15/09
02/22/09
02/28/09
03/07/09
03/14/09
03/22/09
03/28/09
04/05/09
Date
A
B
C
LOW TIDE
(d)
Dissolved Oxygen saturation (%)
Date
A
B
C

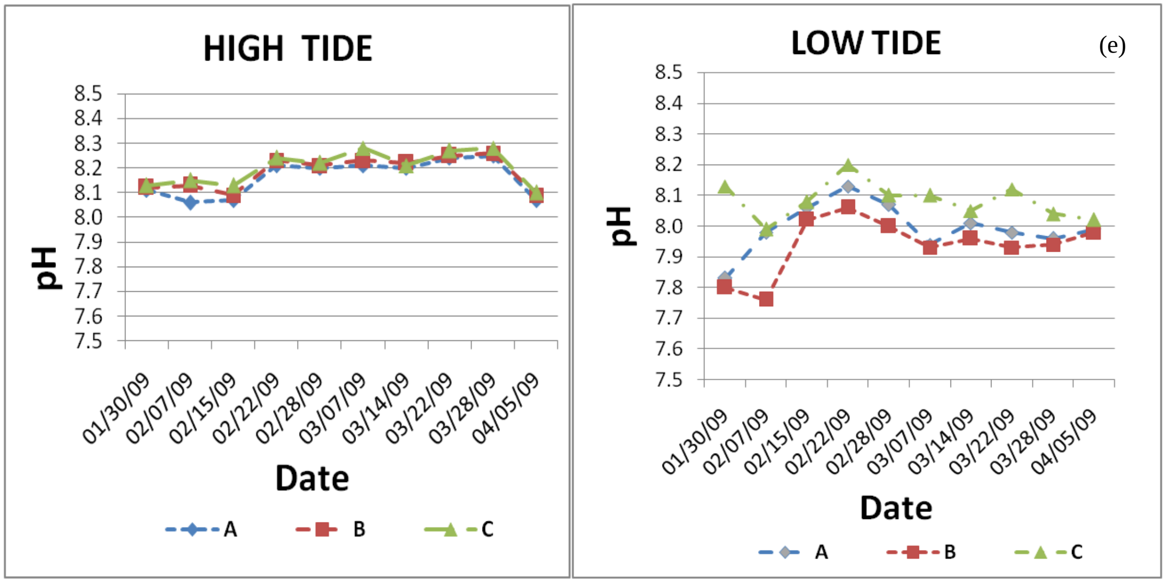


Figure 6. Weekly physicochemical parameters:(**a**) temperature (°C), (**b**) salinity (PSU) and (**c**) dissolved oxygen concentration (mg L^{-1}), (**d**) dissolved oxygen saturation (%) and (e) pH recorded high and low tides along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Table 4. Shell dimensions (mean ± S.D.) of *Isognomon alatus* in control and acidification tanks.

Shell dimensions	Control	Acidification
Initial buoyant weight (g)	0.838 ± 0.29	0.816 ± 0.38
Final buoyant weight (g)	0.829 ± 0.29	0.806 ± 0.39
Initial weight (g)	27.78 ± 3.64	28.44 ± 2.57
Final weight (g)	27.28 ± 3.64	27.75 ± 2.75
Initial height (mm)	27.08 ± 1.05	28. 44 ± 1.30
Final height (mm)	27.08 ± 1.05	27.72 ± 1.52
Initial length (mm)	25.82 ± 3.91	26.67 ± 3.33
Final length (mm)	25.82 ± 3.91	25.31 ± 3.54
Initial thickness (mm)	4.56 ± 0.67	4.36 ± 0.70
Final thickness (mm)	4.56 ± 0.67	4.37 ± 0.72

S.D. = Standard deviation.

Table 5. Shell dimensions (mean ± S.D.) of *Isognomon alatus* cultured along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Shell dimensions	Stations		
	A	B	C
Initial buoyant weight (g)	1.30 ± 0.00	1.31 ± 0.22	1.53 ± 0.38
Final buoyant weight (g)	1.47 ± 0.10	1.36 ± 0.25	1.55 ± 0..34
Initial height (mm)	35.22 ± 0.50	32.72 ± 0.40	34.94 ± 1.09
Final height (mm)	34.29 ± 0.01	31.92 ± 1.12	33.93 ± 0.10
Initial length (mm)	32.50 ± 1.0	31.80 ± 1.90	34.30 ± 3.00
Final length (mm)	33.50 ± 0.5	33.30 ± 0.00	31.50 ± 0.90
*Thickness (period 2)	5.09 ± 0.88	5.18 ± 0.91	5.36 ± 1.08
*Final thickness (period 3)	5.08 ± 0.92	5.19 ± 0.95	5.32 ± 1.11

S.D. = Standard deviation.* Growth period 2 (09/03/09) and **Growth period 3 (05/04/09).

Table 6. Coefficient of correlation (R^2) for shell morphometric analysis of *Isognomon alatus* cultured in control and acidification tanks.

Shell morphometric relationships	Coefficient of correlation (R^2)			
	Control		Acidification	
Power regression between two shell morphometric variables	**Before**	**After**	**Before**	**After**
Buoyant weight (g)/length (mm)	0.269	0.267	0.746	0.740
Buoyant weight (g)/height (mm)	0.457	0.457	0.583	0.660
Buoyant weight (g)/thickness(mm)	0.565	0.565	0.811	0.810
Height (mm)/length (mm)	0.511	0.511	0.319	0.615
Height (mm) /thickness (mm)	0.402	0.402	0.537	0.362
Length (mm) /thickness (mm)	0.302	0.302	0.480	0.472

Average (± standard deviation) growth rate of *I. alatus* ranged from 0.26 ± 0.90 to 1.29 ± 0.77 mg $g^{-1}day^{-1}$ (station A), 0.33 ± 0.46 to 1.47 ± 0.57 mg $g^{-1}day^{-1}$ (station B) and -0.26 ± 2.01 to 0.56 ± 0.40 to mg $g^{-1}day^{-1}$ (station C) (Figure 8c; Table 9). Overall, a positive growth rate of *I. alatus* occurred in the field experiment; 0.55 ± 0.65 mg $g^{-1}day^{-1}$ (station A), 0.89 ± 0.43 mg $g^{-1}day^{-1}$ (station B) and 0.20 ± 0.70 mg $g^{-1}day^{-1}$ (station C) (Table 9). There was no significant ($p > 0.05$) (Table 9) variation in the growth rate (mg $g^{-1}day^{-1}$) of *I. alatus* among the stations (A, B, and C), although observation of negative growth rate for a replicate culture of *I. alatus* at station C (Figure 8c).

One oyster died in the acidification tank, while in the field experiment, mortality was three oysters from stations A and B.

DISCUSSION

Coastal areas such as estuarine are very dynamic ecosystems mostly affected by climate and environmental changes (Braga et al., 2020; Ramajo et al., 2020). Shellfish plays an essential ecological role in coastal ecosystems (Braga et al., 2020; Ramajo et al., 2020). Coasts and their marine biota are exposed to significant environmental heterogeneity due to natural drivers and anthropogenic stressors (Braga et al., 2020; Ramajo et al., 2020). The impact of anthropogenic ocean acidification on marine life is still unclear (Matoo et al., 2020; Yokoyama et al., 2020).

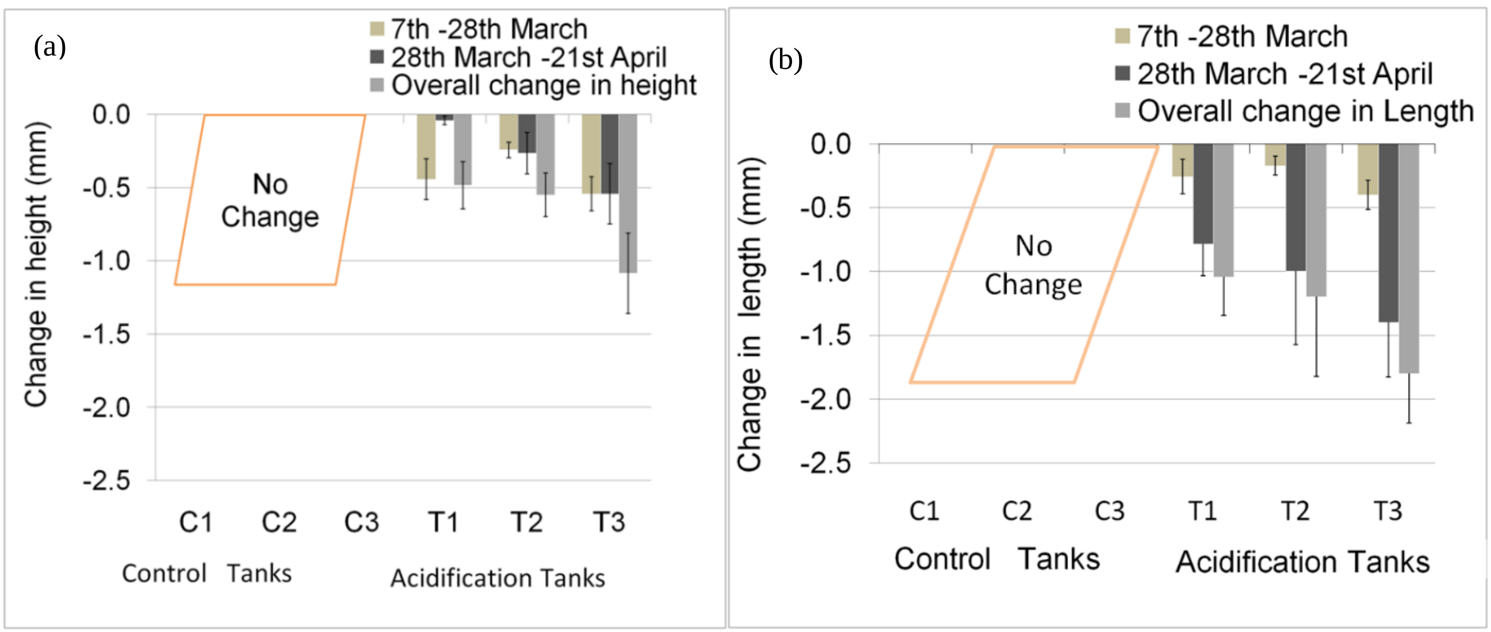
The seawater chemistry of the laboratory changes in pH due to manipulated CO_2 levels (Table 1). The measurement of physicochemical variables in experimental tanks in the laboratory except pH is typical of Atlantic coastal water conditions for that season (Figure 5a–d). The only variation is the pH in control and acidification tanks (Figure 5e) (Table 2). The average pH difference was between ~0.2 to 0.3 units, which corresponds to the lowering of surface ocean seawater pH anticipated in 2100 (Jokiel et al., 1978; Spencer, 1989; Herler and Dirnwöber, 2011).

The physicochemical parameters (Table 3) measured Mangrove Bay Estuary reflect variation in the tidal activity (Figure 6a–e). Many coastal estuarine bays exhibit natural variation in seawater pH due to diurnal changes in

Table 7. Coefficient of correlation (R^2) for shell morphometric analysis of *Isognomon alatus* cultured along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Shell morphometric relationships	Coefficient of correlation (R^2)					
	Stations					
	A		B		C	
	Power regression between two shell morphometric variables					
	Initial	Final	Initial	Final	Initial	Final
Buoyant weight (g)/length (mm)	0.799	0.782	0.620	0.688	0.656	0.839
Buoyant weigh (g)/height (mm)	0.649	0.659	0.688	0.857	0.752	0.755
Buoyant weight (g)/thickness (mm)	N/A	0.542	N/A	0.673	N/A	0.865
Height (mm) /length (mm)	0.418	0.564	0.640	0.743	0.689	0.865
Height (mm)/thickness (mm)	N/A	0.226	N/A	0.441	N/A	0.573
Length (mm)/thickness (mm)	N/A	0.356	N/A	0.264	N/A	0.723

N/A = Not available.



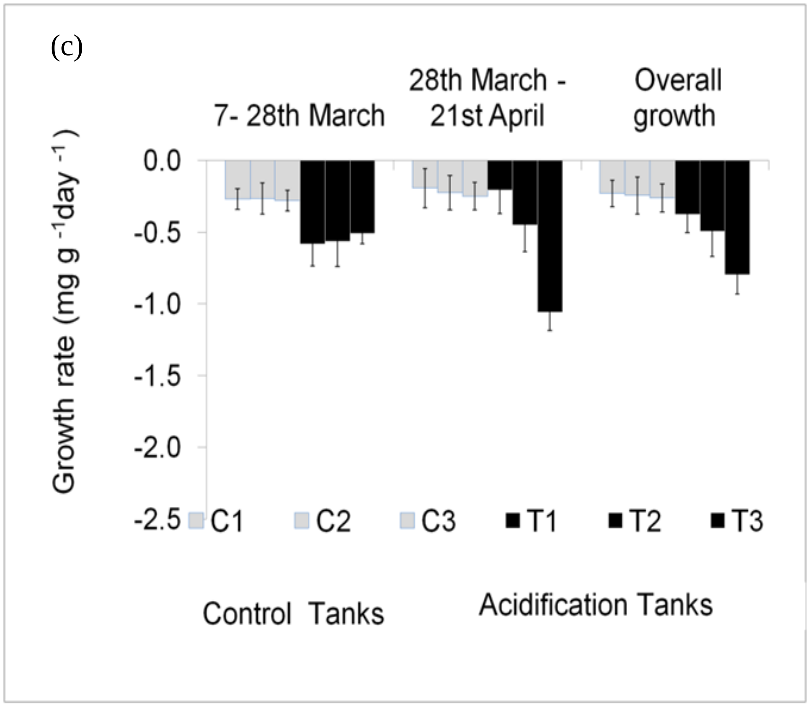


Figure 7.Changes in shell parameters: (**a**) height (mm) (**b**) length (mm) and (**c**) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* over the 45 days experimental period. The period between sampling days were 21 and 24 days. Black bars are control tanks (C1 – C3), and white bars are Acidification tanks (T1 – T3). Error bars±SE, n = 7 except tank T3, in which one oyster died.

Table 8. Average (± S.D.) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* cultured in control and acidification tanks.

Growth period	Culture date	Control	Acidification	Two-sample t-test
1	7 – 28 March, 2009	-0.27 ± 0.30	-0.55 ± 0.40	P < 0.05
2	28 March – 21 April, 2009	-0.25 ± 0.32	-0.57 ± 0.59	P < 0.05
	Overall growth	-0.26 ± 0.23	-0.56 ± 0.36	P < 0.05

the tidal regime and photosynthetic activity (Twilley et al., 1992; Yates et al., 2007). The temperature recorded (16.74 to 21.24°C) is typical of tropical coast weather from January to April. Water depth and volume may influence not only the estuarine water temperature but also season weather changes. During high tide, water volume in Mangrove Bay Estuary increases water depth with cooler temperatures. However, the estuarine water volume may decrease during low tide with a subsequent decrease in water depth, resulting in a shallow-water depth with much exposure to sunlight, then characterized by warmer temperatures. Salinity is maximum at high tide and minimum at low tide (Figure 6b). Tidal influence plays a critical role in estuarine salinity (La Peyre et al., 2016).There is no river flow, but groundwater sources may supply freshwater in Mangrove Bay Estuary. There is an increased flow of open seawater into the estuary during high tide, leading to increased salinity. Dissolve oxygen (concentration and saturation) (Figure 6c-d) and pH (Figure 6e) decreases with rising tidal with a minimum at high tide. At the same time, these parameters increase with the full surge with a maximum at low tide.

The loss of weight of *I. alatus* cultured in the laboratory suggests suppression in a stressful environment (Table 4). Food supply is the primary environmental factor that influences oyster growth and is affected by factors such as temperature, population density, and turbidity (Brown, 1986; Baillie and Grabowski, 2019; Campbell and Hall, 2019). The oyster growth rate is mainly regulated by food supply with temperature and salinity as secondary factors (Brown, 1988; La Peyre et al., 2016). Other possible environmental variables such as water temperature,

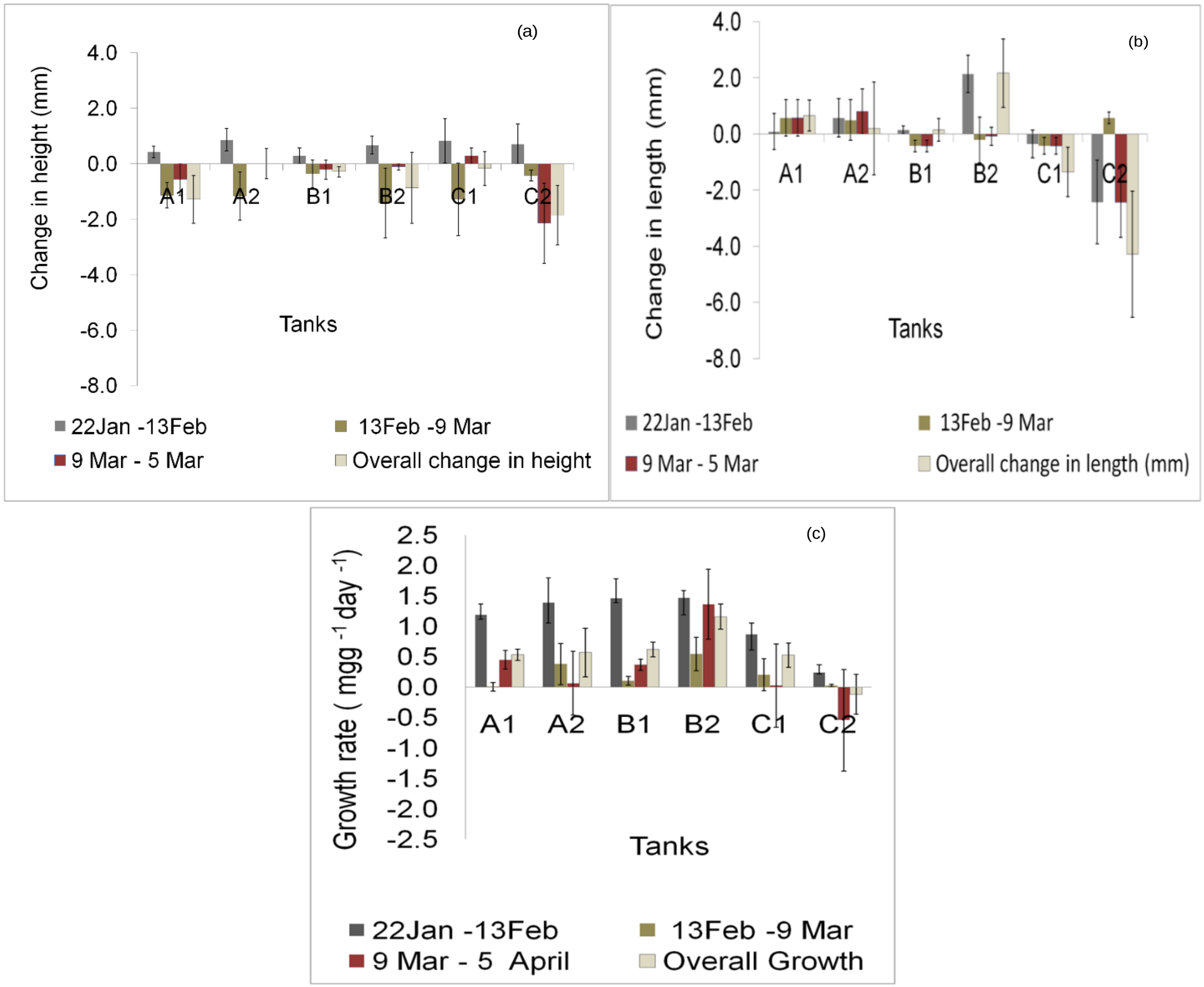


Figure 8. Changes in shell dimensions: (**a**) height (mm), (**b**) length (mm) and (**c**) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* cultured in Mangrove Bay Estuary, Bermuda. Each station has a replicate (A1, A2, B1 B2, C1, C2). Error bars indicate standard errors.

Table 9. Average (± S.D.) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* in the field cultured at Mangrove Bay Estuary, Bermuda.

Growth period	Culture date	A	B	C	One-way ANOVA
1	22 January – 3 March,2009	1.29 ± 0.77	1.47 ± 0.58	0.56 ± 0.40	P > 0.05
2	13 February – 9 March, 2009	0.19 ± 0.55	0.33 ± 0.46	0.11 ± 0.38	P > 0.05
3	9 March – 5 April,2009	0.26 ± 0.90	0.87 ± 0.88	-0.26 ± 2.01	P > 0.05
	Overall growth	0.55 ± 0.65	0.89 ± 0.43	0.20 ± 0.70	P > 0.05

S.D. = Standard deviation.

salinity, and oxygen are dynamic and may affect oysters' growth and survival (Dekshenieks et al., 1993; La Peyre et al., 2016; Dong et al., 2018). The shell dimensions (Table 5) of *I*. *alatus* cultured in the field culture reflect optimum conditions. The loss of weight of *I*. *alatus* in control tanks and acidification tanks (Figure 7a–c) could be due to a decrease in organic tissue due to insufficient food availability. There exists an association between two shell morphometric variables (Table 6 and 7). The drastic changes in shell dimensions (height, length, weight loss) (Table 4) and growth rate of *I*. *alatus* (Table 8) cultured in acidification tanks (Figure 7a–c) could be due to multiple environmental stressors including reduced metabolic activity, insufficient food source, and shell thinning in CO_2 acidified seawater (Figure 7a–c). Therefore, exposure of flat tree oysters to low pH could significantly decrease skeleton weight and damage the shell structure.

In this study, the shell length of *I*. *alatus* measured ranged from 27.08 to 28.44 mm in the laboratory while 31.92 to 35.22 mm in field culture. The shell height of *I*. *alatus* ranged from 25.82 to 27.72 mm in the laboratory but 31.80 to 34.30 mm in the field culture. The adult *I*. *alatus* ranged from 75 mm to 95 mm in shell length and 40 - 50 mm in shell height (Mikelsen and Bieler, 2008), although individuals as large as 90 mm in size have been encountered (Siung,1980).

In contrast, *I*. *alatus* showed a gain in weight and positive growth exclusive of growth period 3 (Table 9) in field culture could be due to favourable environmental conditions, despite exposure to low pH (~7.4) levels during low tide (Figure 8a–c). Therefore, *alatus* cultured in the natural field showed tolerance to low pH exposure during the low tide. The results demonstrated that flat tree oyster's response to future CO_2 levels is complex. Other environmental l factors such as food availability and diurnal variability of the tidal activity in an estuarine environment are also potential factors that can compound ocean acidification impacts on marine animals.

The weight loss and shell thinning of *I. alatus* observed in this study (Table 10) agree with the effects of lowering pH on marine animals when cultured in manipulated CO_2 acidified seawaters (Table 10) (Talmage and Gobler, 2010; Yu et al., 2011). The lowering of pH reduced the growth of *Mytilus edulis* (Berge et al., 2006). In pH 6.7, small mussels (11 mm mean length) showed low growth rates compared to control treatments, and large mussels (21 mm mean length) did not grow at all. A reduction in seawater pH was responsible for growth suppression, shell dissolution, tissue weight loss, and feeding activity suppression in three species of commercial bivalves (*Ostrea edulis*, *Crassostrea gigas,* and *Mytilus edulis*) (Bamber, 1990). A long-term lowering of seawater pH caused a reduction in haemolymph pH, buffered by the dissolution of the shell ($CaCO_3$), lower metabolic rate, and increased degradation of protein in mussel, *Mytilus galloprovincialis* (Michaelidis et al. 2005). Ocean acidification resulted in physiological stress in the adult mussel, *Mytilus chilensis* (Diaz et al., 2018).

There are biological consequences of changing ocean pH (Widdicombe and Spicer, 2008; Convey and Peck, 2019; Emanuel et al., 2020; Stokowski et al., 2020), withadverse effects on physiological processes in marine organisms (Fabry et al., 2008) and ecosystems structure and function (IPCC, 2007; Widdicombe and Spicer, 2008; Stokowski et al., 2020). There are detrimental effects of acidic waters on blue mussel, *Mytilus edulis* (Berge et al., 2006; Bibby et al., 2008) gastropoda, *Littorina Littorea* (Bibby et al., 2007), carpeted-shell clams, *Venerupis decussata* (Bamber, 1987; Bamber, 1990) through a rise in projected pCO_2 levels (Feely et al., 2009; IPCC, 2013; Doney et al., 2015). The calcification rates of the mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas)* declined with increasing pCO_2 (Berge et al., 2006; Gazeau et al., 2007). There was a decrease of 25 and 10% in mussel and oysters, respectively, at pCO_2 levels anticipated by the end of this century (~740 ppm, IPCC IS92a scenario) (Gazeau et al., 2007; IPCC, 2007, 2014, 2018). The reduction in seawater pH leads to growth suppression, shell dissolution, tissue weight loss, and feeding activity suppression of *Mytilus edulis* exposed to future seawater conditions (Bibby et al., 2008). The increased pCO_2 of seawater projected to occur by the year 2300 (pH 7.4) will severely impact the early development of *Crassostrea gigas* (Kurihara et al., 2007). However, there is no significant effect of 2000 ppm CO_2 (pH 7.4) treatments on the fertilization success of *Crassostrea gigas* and *Mytilus galloprovincialis* from Japan (Kurihara et al., 2009). There was no significant effect of future levels of ocean acidification (−0.35 pH unit change) on sperm swimming speed, sperm motility,

Table 10. Ocean acidification effect on culture of marine organisms.

Species	Cultured medium (pH, CO_2, and pCO_2 levels)	Duration	Effect	Source
Isognomon alatus (Gmelin, 1791) (Flat tree oyster)	7.8 – 7.9 (*Acidification-low pH; ~ Three levels of CO_2 = T1 = 193 ppm, T2 = 390 ppm, and T2 = 766 ppm) (Laboratory culture).	45 days	Reduced weight, shell thinning, and significant negative growth rate.	This study
	8.0 – 8.2 (Control-ambient seawater pH) (Laboratory culture)	45 days	Weight loss and significant negative growth rate.	
	7.76 – 8.28 but ~ 7.4 during low tide (Field culture)	45 days	Increase in weight and positive growth. rate	
Tritia reticula (Linnaeus, 1758) (Intertidal gastropod)	8.08 (Control; ambient seawater pH) 7.65 – 7.88 (Low pH; acidified with CO_2)	2 months	Shell repair, with a full repair rate observed in 75% of individuals.	Yokoyama et al. (2020)
Mytilus edulis (Linnaeus, 1758) (Blue mussel)	pCO_2 levels = ~ 400 vs. 800 µatm at control and elevated temperatures (10 vs. 15 °C)	-	Ocean acidification did not affect the metabolism of adult Mytilus *edulis*	Matoo et al. (2020)
Limacina helicina (Philipps,1774) (Plantonic sea snail)	7.78	30 days	28% decrease in calcification rate.	Comeau et al. (2009)
Crassostrea gigas (Thunberg, 1793) (Pacific oyster)	7.4	48 h	Inhibited larval development	Kurihara et al. (2007)
Mytilus edulis and *Crassostrea gigas*	740 ppm	2 h	A decrease in calcification 25% and 15%, respectively.	Gazeau et al. (2007)
Mytilus galloprovincialis (Lamarch, 1819) (Mediterranean mussel)	7.3	3 months	Significant decrease in growth rate.	Michealidis et al. (2005)

CO_2 = Carbon dioxide gas; pCO_2 = Partial pressure of carbon dioxide; acidification tanks (T1-T3).

and fertilization kinetics in a population of oyster, *Crassostrea gigas* (Havenhand and Schlegel, 2009). The acidified seawaters disrupt mangroves ecosystems and estuarine organisms; these will indirectly contribute to economic and societal implications.

Some marine fauna showed resilience to acidified water. When the gastropod, *Tritia reticulate* were cultured in high CO_2, it resulted in shell damage without physiological stress (Yokoyama et al., 2020). Individuals of *Tritia reticulate* were exposed to control ambient pH of 8.08 (control) and low pH scenarios (pH 7.65 and 7.88). After two months of exposure, all individuals showed shell repair, with a full repair rate observed in 75% of individuals (Yokoyama et al., 2020). Ocean acidification did not impact the metabolism and enzyme activities in the blue mussel, *Mytilus edulis*, although the temperature does (Matoo et al., 2020). The blue mussels (*Mytilus edulis*) showed changes in physiological filtration and cilia beat function when cultured increased dissolved carbon dioxide (Meseck et al., 2020). Estuarine is a complex dynamic system, and more than pH modulates the physiological flexibility of mussel, *Perumytilus purpuratus* populations (Ramajo et al., 2020).

I. alatus is a marine species with a potential for aquaculture to increase shellfish supply. The shell of *I. alatus* provides an area of the substrate for other species living in mangrove communities (for example stone crab, *Menippe mercenaria*, gastropod, *Batillaria minima,* and the Coffee bean snail, *Melampus coffeus*) (Patrick, 1988; Thomas and Dangeubun, 1994). The species plays a critical role in the uptake and recycling of nutrients

(a) (b)

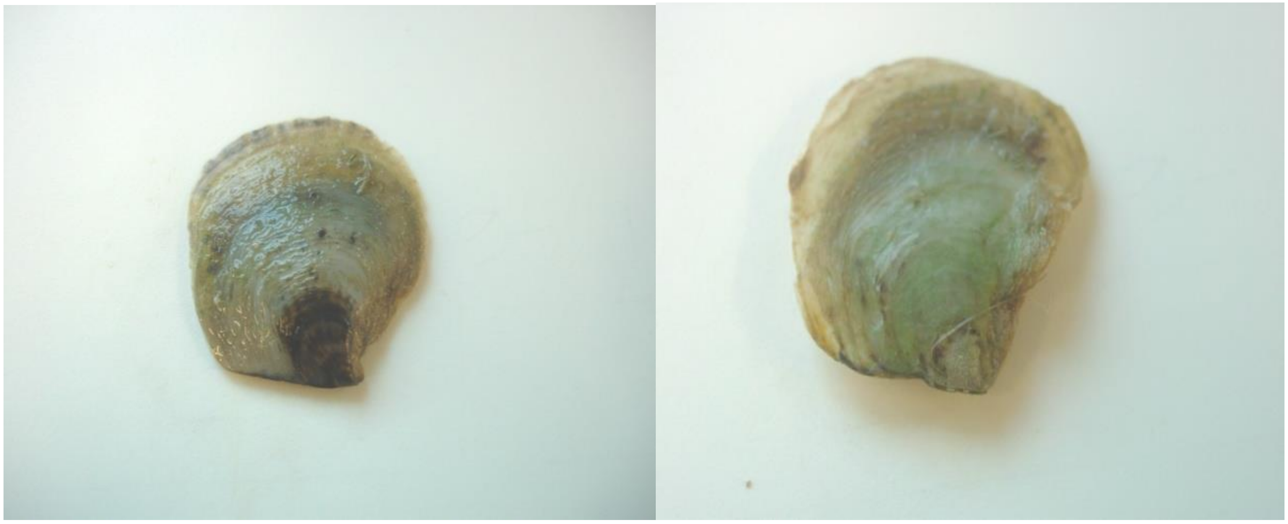


Figure 9.*Isognomon alatus* exposed to (a) ambient seawater pH (pH 8.1 – 7.9) and (b) altered seawater pH (pH 7.8 – 7.9), indicating shell thinning.

in the mangrove ecosystem due to their filter-feedings habits (Gutiérrez et al., 2003; Wilk and Bieler, 2009; Suarez-Ulloa et al., 2019). The species is also used to concentrate pollutants such as heavy metal concentration (e.g., Cu, Pb, Zn, Cd), thereby help to improve coastal water quality, especially in Venezuela, the Caribbean, and the Dominican Republic (Jaffe et al., 1998; Sbriz et al., 1998; Saed et al., 2001; Leal et al., 2019).

I. alatus is an essential food source for other marine organisms in mangrove estuaries and various sea birds (Gutiérrez et al., 2003; Suarez-Ulloa et al., 2019). The species act as a habitat for many small benthic bivalves (e.g., *Melampus coffeus*) that live on the shells and within the byssus threads that hold the oyster onto a substrate (Thomas and Dangeubun, 1994). The species act as ecosystem engineers to maintain coastal estuarine environment biodiversity, sediment stability, nutrient recycling, and safety (Thomas and Dangeubun, 1994; Emanuel et al., 2020). Ocean acidification will affect coastal ecosystems and their resources, such as mangrove bays that provide a substrate for marine benthic organisms (Zablocki et al., 2011; Linto et al., 2014; Leal et al., 2019). This study provides evidence that the lowering of seawater pH due to anthropogenic absorption of CO_2 projected to occur during this century may affect the shell dimensions and growth rate of *Isognomon alatus.* Environmental influence on flat tree oyster is critical for a better understanding of their response to lowering pH.

Conclusion

Flat tree oysters are important benthic fauna in the mangrove ecosystem, serving as a substrate for bottom-dwelling animals. The species are filter feeders supporting nutrient recycling and potential bioindicators for coastal pollution. Short-term bivalves culture in projected future pH conditions is critical to understand how the species will adapt to projected lowering pH due to increased CO_2 concentrations in seawater. *Isognomon alatus*, the flat tree oyster, exhibited much weight loss and a negative growth rate at pH values (7.8 – 7.9) expected by the year 2100 compared to growth rates at present ambient pH values (8.1 – 8.2) (Figure 9). The lowered pH effect (also known as ocean acidification) on the flat tree oyster includes weight loss, shell thinning, dissolution, and mortality. The weight loss and negative growth could be due to insufficient food availability in the experimental tanks. However, a higher decreased weight and negative growth of *I. alatus* cultured in acidification tanks are due to a disturbance system caused by the manipulated CO_2 in the seawater and the lack of nutritional food supply.

In contrast, *I. alatus* cultured along a natural variability of pH gradient due to tidal cycle in Mangrove Bay Estuary showed weight gain and positive growth, despite exposure to low pH (~7.4 value) during low tide. Overall, the results suggest that lowering pH could negatively impact shellfish, such as oysters, with potential dramatic coastal ecosystems changes in structure, function, and services. The study provides evidence of ocean acidification effect on flat tree oyster, commonly attached to mangroves in the coastal estuarine system with natural variation pH due to tidal activity. The findings are useful for modeling biogeochemical changes in coastal ecosystems. Further investigation on marine bivalves' long-term exposure to low pH and environmental controls is necessary to understand their growth under changing CO_2 seawater. Therefore, *I. alatus* under increased CO_2 conditions is critical for their conservation in projected climate change impacts on the coastal environment such as mangroves system and protecting life below water

(Sustainable Development Goal 14).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impact of oil installations on groundwater resources in Bongor Basin, Republic of Chad

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This study examines concentration of pollutants that can contaminate soil, surface water and groundwater in the Bongor Basin. The analysis of thirty soil samples from six oil sites: Koudalwa, Ronier, Ndoubadana, Narenang, Croisement Baobab and Croisement Ridina was carried out using standard laboratory testing methods of chemical elements and pollutants. Results reveal that Nickel has a maximum concentration above the acceptable threshold of INERIS standard of France (2-60 mg/kg). Copper present concentrations above the Cameroonian standard of SONARA site (10 - 30 mg/kg), Cadmium has a content above the INERIS standard of France (0.2-0.7 mg/Kg) and Cameroonian standard of SONARA site (1 - 2 mg/kg). Phenol was present in all samples with very high concentration values compared to Canadian class A and B standards (0.1-1 mg/kg) and Cameroonian standard of SONARA site (0.2-1 mg/kg).

Keywords: Oil installations, pollutants, groundwater resources, basin, Bongor.

INTRODUCTION

Soil and water contamination by hydrocarbons is of concern because of the toxic nature of hydrocarbons for all forms of life. Hydrocarbons, metals and other chemical compound associated with oil development and facilities detected in soil and environment come from water produced during hydrocarbon production. This water consists of reservoir water brought to the surface with hydrocarbons, as well as condensation and reinjection water for pressure maintenance and production optimization (UNEP, 1997). Thus, all chemical elements associated with hydrocarbons and those contributing to its exploitation might be dispersed on the ground, and dissolved in water or float freely on the surface of the water. These chemical elements are real problems for the environment (Aniefiok et al., 2016). In the history of hydrocarbon development, several cases of oil and chemical spills have been recorded (Jacqueline and Emilio, 2017). Increased land pressure, urbanization, the development of oil and industrial facilities, and the dramatic deterioration of the environment associated with various spills are often source of conflict between residents, operating companies and existing governments

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(Bruno and Pascale, 2000). In Chad, companies recorded several cases of oil and chemical spills in oil fields (Anonymous 1, 2018). Other research also raised concern about oil spill problems in the Doba Basin (ECMG, 2009). Concerning Bongor Basin, according to the Initiative for Transparency in Extractive Industries in Chad (ITIE, 2014), environmental disputes have been an arm wrestling between the government and oil companies. These disputes are due to flagrant violation of environmental standards by intentional spills of crude oil into open pits and trenches in the Koudalwa Region (Croset, 2015). Despite various notifications, these bad practices influencing the environment of these localities have not stopped. Buried crude oil spilled during exploitation in pits and on the ground might likely result in soil, surface water and groundwater contamination. In view of reports on oil spills, chemicals, and all oil practices, the receptacle for all kinds of waste generated by oil activities in this environment is the soil. It plays a decisive role in the flow or dispersion of these harmful substances and is the main point of dispersal of environmental pollutants (Wilcke, 2000). In order to know the state of the environment in Bongor Basin, this research carried out focuses on the determination of concentration of pollutants that could contaminate soil, surface water and groundwater. Samples were taken from six oil sites in Bongor Basin.

MATERIALS AND METHODS

Area of study

Bongor Basin is located southeast of N'Djamena (Chad), between 15.15 and 17.50° East longitude and between 9 and 11.25° North latitude with surface area of about 105767 km^2. It is located 180 km from the starting point of Komé pipeline. It is located in a transition zone between the humid tropical climate and the dry tropical Sahelian climate, marked by an alternation between wet and dry seasons. Rains begin with some precipitation in April and last until October with maximums precipitation in July, August and September (PANA, 2009; Atlas TCHAD, 2013; Anonymous 3, 2017). The average lowest temperature is in January (24.6 degrees), the average and maximum temperature is 40 and 47 degrees, respectively. We observe peak temperature in April. The minimum temperature ranges from 10 to 20 degrees in December (ATLAS TCHAD, 2013; Anonymous 3, 2017).

This zone is highly dependent on physical and chemical phenomena, linked to changes in temperatures and the amount of rain that can trigger altered and mineralized reactions (Anonymous 2, 2017). Soils that predominate in this area are tropical ferruginous soils formed on silica sands or clay sands (Anonymous 2 and 3, 2017). They are associated in some parts with halomorphic soils and in other places with hydromorphic soils. Vertisols or hydromorphic soils have poor internal drainage. They are flooded during the rainy season for several months (Nadjiam, 2013; Anonymous 3, 2017). Bongor Basin Oil Fields are located north of the Chari River and south of the Logone River. These two rivers are the main permanent rivers in CHAD. Chari River which is near the study area has an average annual flow rate of 1230 m^3/s, the maximum netflow rate measured to be 5160 m^3/s, and the average annual runoff is about 38.8 billion m^3 (Anonymous 2 and 3, 2017). This river has its origin from Central African Republic, fed by Baminguiand then Bangoro streams. Its main tributaries are Barh Aouk, Barh Keita and Barh Salamat on the right bank; Bahr Sara and Ba Illi on the left bank (PANA, 2009; ATLAS TCHAD, 2013; Anonymous 3, 2017).

The vegetation of the site is a Sudanese Savanna tree supported by sandy-textured soils. Forest formations are more or less dense to combretaceous (Anonymous 3, 2017). There are also clear forests, shrub savanna, fallows, grasslands and gallery forests along temporary streams (PANA, 2009; ATLAS TCHAD, 2013; Anonymous 3, 2017). The geology of Chad dominated by the Lake Chad Basin is a sedimentary basin forming in Mesozoic times and filled, in depth, with tertiary-age sandstone and clay sands from the continental terminal; superimposed by the Pliocene-quaternary sandy age/clay deltaic sediments and lacustrine sediments. Bongor Basin is within the lower end of base Oligo-Miocene at Neogene located in large parts of southern Chad. It consists of alternating layers of sandstone, clay sand and clay generally at depths of 400 to 700 m, below the quaternary-Pliocene formation shown on the geological map in Figure 1 (Ngatcha et al., 2008; UNICEF, 2010). Aquifers in this area include three main aquifers: an upper, unconsolidated Quaternary/Pliocene aquifer of lacustrine and deltaic deposits (the Chad Formation); a Tertiary sequence underlying this (the Continental Terminal formation) (GWP, 2013).

Sampling method

The approach used for sampling is the hypothetical approach, with a control sample that amounts to a subjective selection of sampling points based on on-site visual recognition. The type of sampling is punctual and redesigned (ISO, PART 2, 1993). The collection points are under oil wells, manifolds, pipelines (Plates 1 to 3), on a space of 30 × 30 m^2 of oil facilities. To ensure good representativeness of samples, fields and or sites are crossed in zig-zag by taking random sub-samples throughout the field area. Each sub-sample consisted of 200g obtained from a 25 cm high stainless steel cylindrical tube core and 50 mm in diameter (ISO, PART 1, 1993). A sample consists of several sub-samples from a collection site and put together. At each locality site, five (05) samples were collected, including a control sample, and 4 samples from the targeted areas or parts of the fields with quite different ground-looking colour. Each sub-sample was stored in a resistant polyethylene bag. The set of sub-samples by site or oil fields put together in a larger plastic bag, placed in a wooden case, and transported to Water and Environment (LABEN) Laboratory at the Faculty of Exact and Applied Sciences at University of N'Djamena in Chad. 30 samples were collected from 72 points visited and identified; 5 samples per oil field or site (Figure 3) are taken from six localities in Bongor Basin (Figure 2): Koudalwa, Ranger, Ndoubadana, Narenang, Croisement Baobab and Croisement Ridina.

Soil samples processing

Soil samples taken from the target points in oil sites were air-dried at room temperature. A follow-up mixture of sub-sample quartage was made, and then a quantity was crushed using a porcelain mortar and pestle; it was sieved using a 100-mesh sieve. 250 g of the soil sieved was stored in suitable polyethylene plastic and numbered according to codes defining their origin. 10 g of previously treated soil sample was diluted in 100 ml distilled water volume, and filtered by the WAH filtration device (2008-2012). Filtrates obtained from samples are stored for laboratory analysis (Plates 1 to 3).

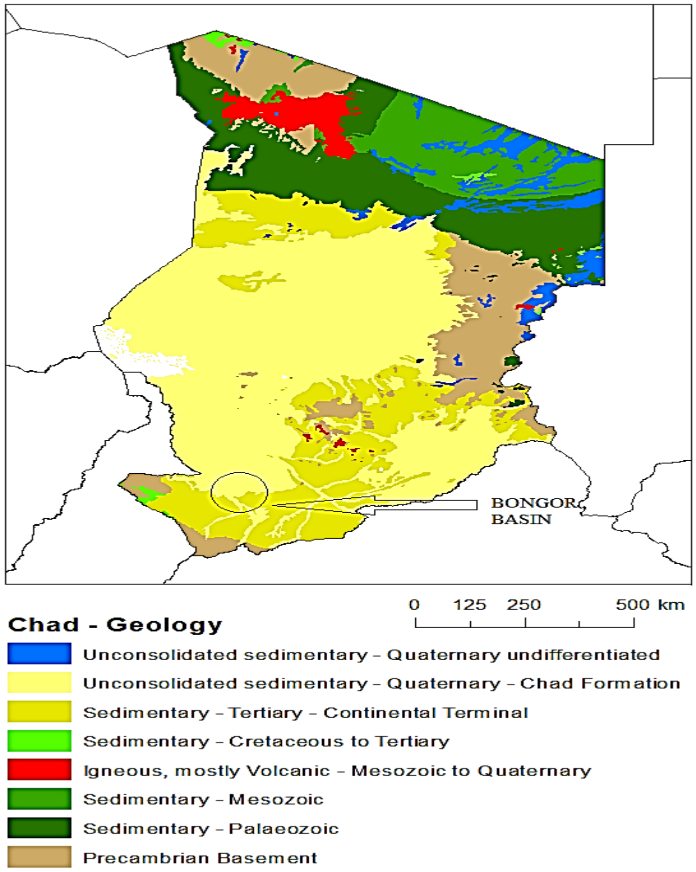

Figure 1. Geology of Bongor basin.
Source: developed from USGS map by Persits et al. (2002).

Plate 1. Oil spill at Croisement Ridina. (b)Well-near sampling points, (a), (c) and (d) drain points.

Plate 2. (a) Sampling points close to pipelines, (b) crude oil gathering stations and processing centre facilities in Koudalwa.

Plate 3. (a) Sample points close to manifolds, (b) newly drilled wells, (c) and (d) abandoned wells at Croisement Ridina.

Chemical analysis methods

Chemical parameters determination methods are presented in Table 1.Concentrations values obtained in mg/L are then converted in mg/Kg of dry matter, according to these formulas:

$$[\text{Metal or chemical element (mg/Kg)} = \frac{\text{metal concentration (mg/L) x sample volume (mL)]}}{\text{[sample weight (kg) x 1000]}}$$

$$[\text{Metal or chemical element (mg/Kg)}] = \frac{\text{[metal concentration (mg/L)x final volume (mL)]}}{\text{[initial sample weight (g)]}}$$

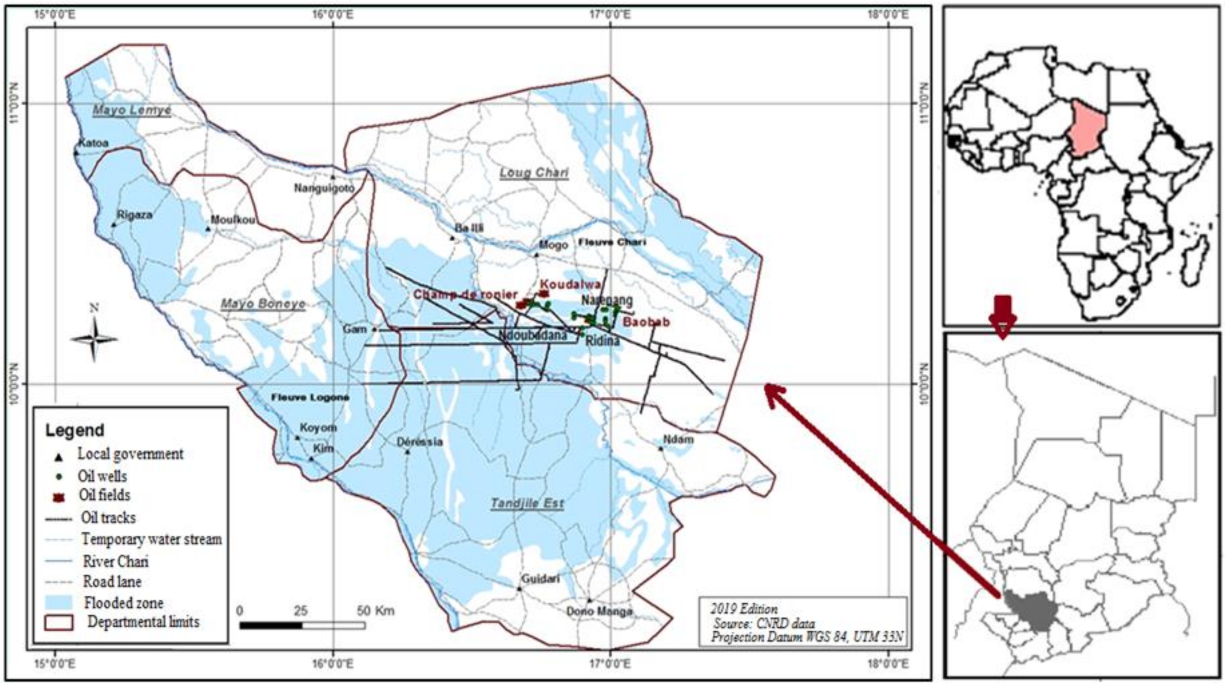

Figure 2. Bongor Oil Basin localization and the six oil sites localities of the study area.

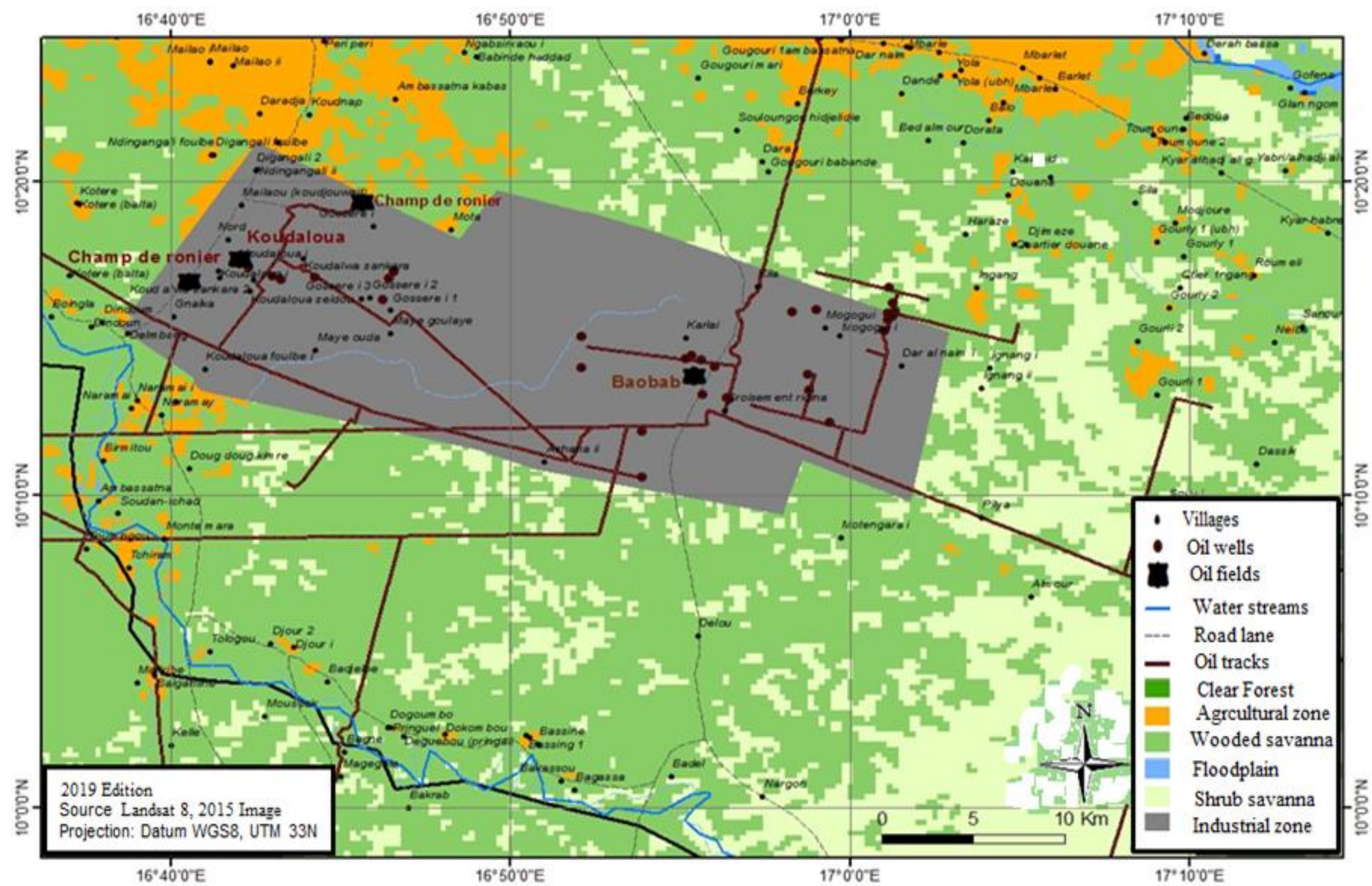

Figure 3. Soil sampling sites in Bongor basin oil fields.

Table 1. Pollutants sought, methods and devices used.

Chemical parameter	Methods et devices used	References
pH	pH-meter EcoScan Ion 5/6 and Cyberscan PH110	WAH (2008-2012)
Heavy metals and metal trace elements		
Chrome (Cr)	WAH Method 8024 (2008-2012). Spectrophotometer UV DR/2400.	WAH (2008-2012)
Zinc (Zn)	8009 method (USEPA Zincon Method Spectrophotometer UV DR/2400.	
Nikel (Ni)	WATANABE's adapted 1-(2-Pyridylazo) -2Naphtol (PAN) method, TALANTA, 21,295 (1974). Spectrophotometer UV DR/2400.	
Copper (Cu)	ISH and KOH method, BUNSEKI KAGAKU, 28,473. Spectrophotometer UV DR/2400.	
Cadmium	UV/VIS-1700PC spectrophotometer (SHIMADZU). The method used is the spectrophotometric determination of toxic elements (cadmium).	
Other chemical elements		
Sulphur	Methylene blue method (USEPA 376.2 and Method 4500-S2-D for wastewater). UV DR/2400 Spectrophotometer (0-0.70 mg/l S) adapted from Standard Methods for the Examination of Water and Wastewater	WAH(2008-2012).
Total nitrogen	UV Spectrophotometer DR/2400 by persulfate digestion with the 10071Test N Tube method	
Toxic chemical elements		
Mercury	10065 method or Cold Vapor Mercury Concentration Method. UVDR/2400 Spectrophotometer	WAH (2008-2012)
Phenol	Accepted 8047 method used by USEPA, 4-Aminoantipyrine Method (distillation required), the procedure is equivalent to USEPA's 420.1 method for wastewater. The UV DR/2400 Spectrophotometer	
Total hydrocarbon	Gas phase chromatography coupled with a flame ionization detector (CPG/FID) Emerson's 700XA gas phase chromatograph is used.	NF EN ISO 9377-2 (Ranking Index: T 90-150), NF EN 14039 and (X 30-405 ranking index) and MA. 400 - C10C50 1.0. 400 - C10C50 1.0.

The results obtained, are analyzed based on control samples by site and standards; it is also in comparison with the maximum, average and minimum values. The pH scale (Figure 4), which gives acidic solutions with a pH of less than 7 and basic or alkaline solutions with a pH greater than 7 (Nutrient Manager, 1996), has been used to measure the acidity and alkalinity of the soil solution. Soil pH is influenced by acidic and basic soil cations (Brady and Weil 1999).

RESULTS AND DISCUSSION

Hydrogen potential

The pH measurements of samples from the different localities of Bongor Basin range from 4 to 9 with an average of 7.39. The average and maximum pH values are within the pH range of alkaline soil, and the minimum value of 4.8 is in the range of acid soil content (Figure 4). Values of pH close to 8 and above, within the alkaline soil range (Figure 4), are obtained from Koudalwa locality (BBS01 and BBS03), Ronier (BBS06 and BBS08), Ndoubadana (BBS11), Narenang (BBS17), Croisement Baobab (BBS22 and BBS23) and Croisement Ridina (BBS26 and BBS30). While the samples with pH values within acid pH interval are from Croisement Ridina locality (BBS27).

Concentration of heavy metals in soils samples from oil field sites

Chromium

The concentration of soil samples from various oil sites or near oil facilities ranges from 0.2 to 9.5 mg/kg with an average of 4.07 mg/kg, a standard deviation of 2.32 and a coefficient of variation of 0.57. These concentrations are within the range of standard values. Sample BBS08 from Ronier locality has low concentration (0.2mg/kg) of chromium (Figure 5). This concentration is as low as that of control sample BBCS10 from the same locality, which has a value of 1.5 mg/kg. The high chromium concentration value (9.5 mg/kg) is from sample BBS26 of Croisement Ridina locality. This concentration is very

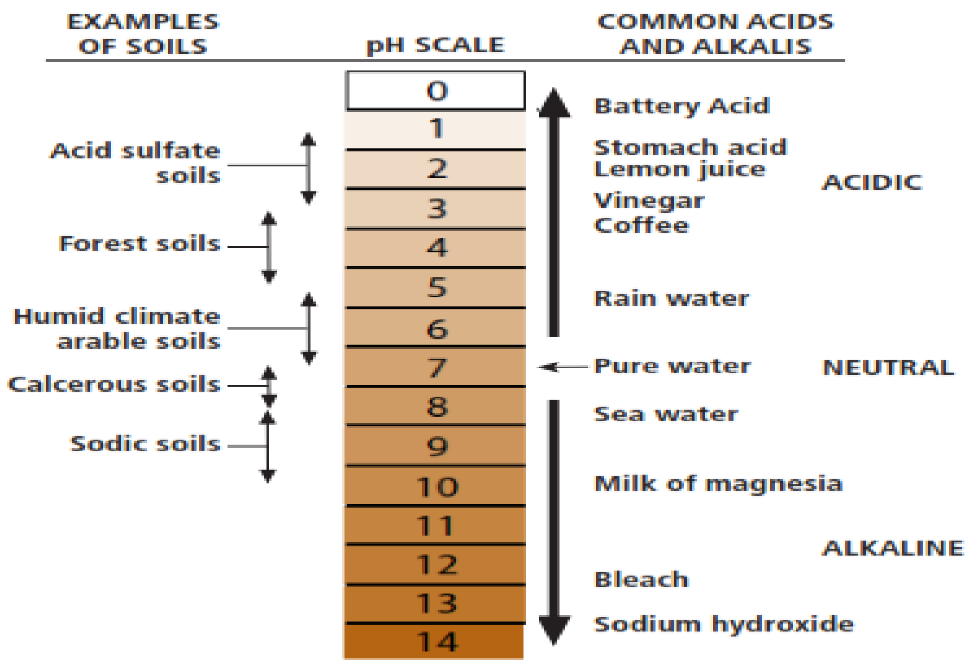

Figure 4.pHscale (Nutrient Manager, 1996. Focus on pH and lime).

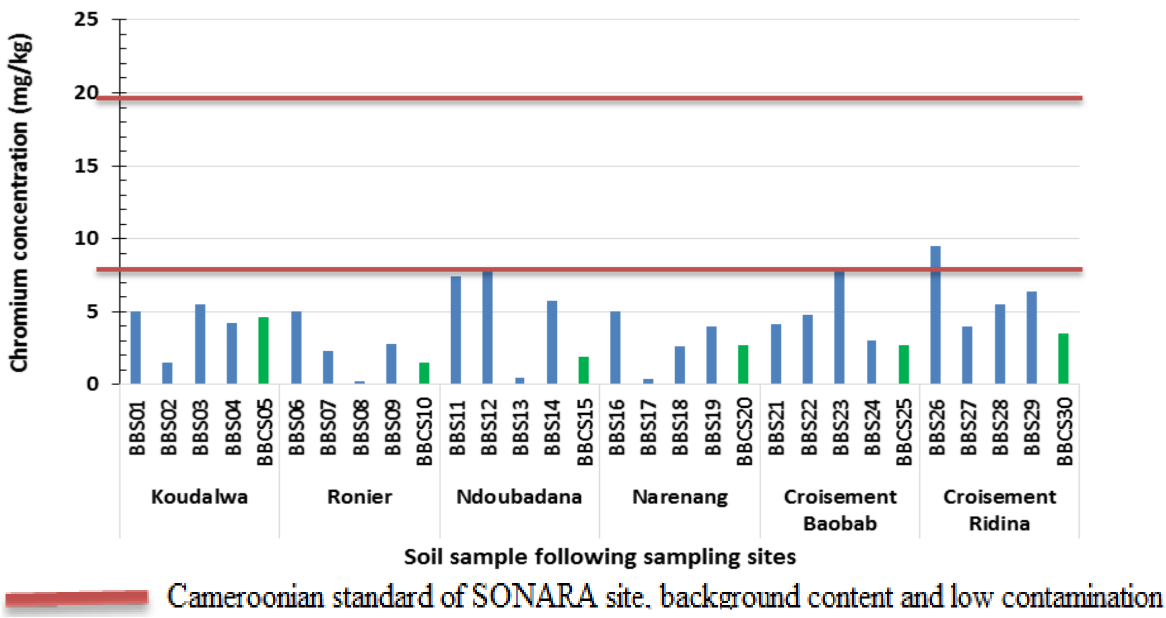

Figure 5. Concentration of Chromium in the soil of oil fields and facilities. BBCS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

high compared to the control sample BBCS30 (3.50 mg/kg) of the same locality (Figure 5).

Nickel

Concentration level of Nikel in soil samples from various oil sites or installations ranges from 0.5 to 102 mg/kg, with an average of 25.67 mg/kg, a standard deviation of 24.57 mg/kg and a coefficient of variation of 0.96, pointing out high levels of Nikel concentration in these oil sites or localities. Some Nikel content values are not within the range of INERIS standard of France (2 -60 mg/kg) and Cameroonian standards of SONARA site,

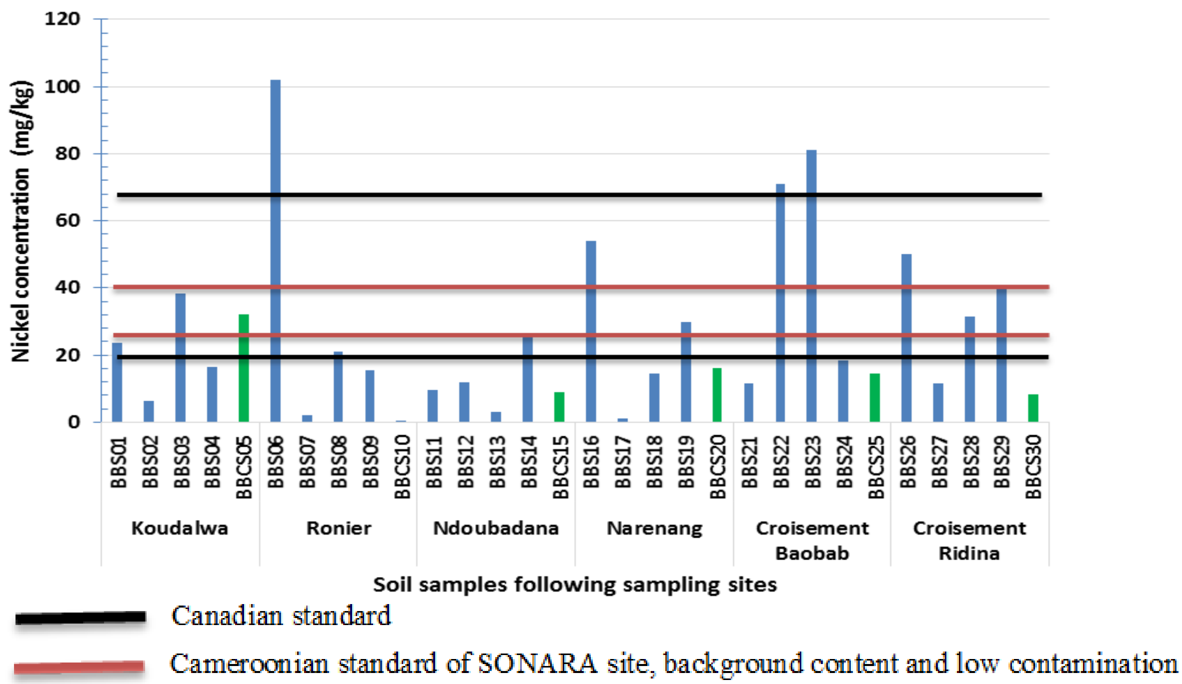

Figure 6. Nickel concentrations in the soil of oil fields and facilities. BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

background content (≤15 mg/kg) and low contamination (15-30 mg/Kg). Concentrations of samples BBS07 and BBCS10 of Ronier locality, sample BBS13 in Ndoubadana locality and sample BBS17 in Narenang locality (Figure 6) have Nikel content values below INERIS standards of France. On the other hand, samples BBS06 from the locality of Ronier, BBS22 and BBS23 from Croisement Baobab locality have values above this standard. Compared to low contamination of Cameroonian standards of SONARA sites, sample BBS03 from Koudalwa locality, sample BBS06 from Ronier, sample BBS16 from Narenang, samples BBS22 and BBS23 from Croisement Baobab locality and samples BBS26 and BBS29 from Croisement Ridina have higher values (Figure 6).

Copper

Copper concentration in the study area ranges from 12.5 to 52 mg/Kg, with an average of 30.91 mg/Kg. The coefficient of variation is 0.34 with the high standard deviation of 10.59 mg/Kg, indicating high presence of copper in these different localities (Figure 7). Copper concentrations of all samples are within the range of Canadian standards (40-500mg/kg) and INERIS standards of France (2-60 mg/Kg). Concentrations of copper within the range of low contamination of Cameroonian standards of SONARA site (10- 30 mg/kg) are observed from samples BBS06, BBS07 and BBS08 of Ronier locality, sample BBS13 of Ndoubadana, samples BBS17 and BBS18 of Narenang, samples BBS21, BBS23 and BBS24 of Croisement Baobab locality (Figure7). However, it is observed from samples BBS01, BBS02, BBS03, BBS04 and BBS05 of Koudalwa locality, samples BBS11, BBS12 and BBS14 of Ndoubadana locality, sample BBS16 of Narenang, samples BBS26, BBS28 and BBS29 of Croisement Ridina, high values compared to low contamination level of Cameroonian standards of SONARA sites (10 - 30 mg/kg). The control sample BBS30 is the only one of low concentration compared to samples from the same locality (Figure 7).

Zinc

Zinc concentration of all soil samples analyzed ranges from 0.5 to 42.7mg/kg with an average of 15.63mg/kg and a coefficient of variation of 0.77. All samples have concentrations which are within the interval of Cameroonian standards (of the low contamination) of SONARA site (15-50mg/kg); they are low and within the range of Canadian standards (110, 500 and 1000 mg/Kg) and INERIS of France (40 - 180 mg/kg). This wide gap and values of zinc concentration with extremely low values compared to Canadian standards might be from the nature of the soil environment and climate conditions. Results have shown that all values of zinc concentration in Koudalwa locality are lower than that of the control

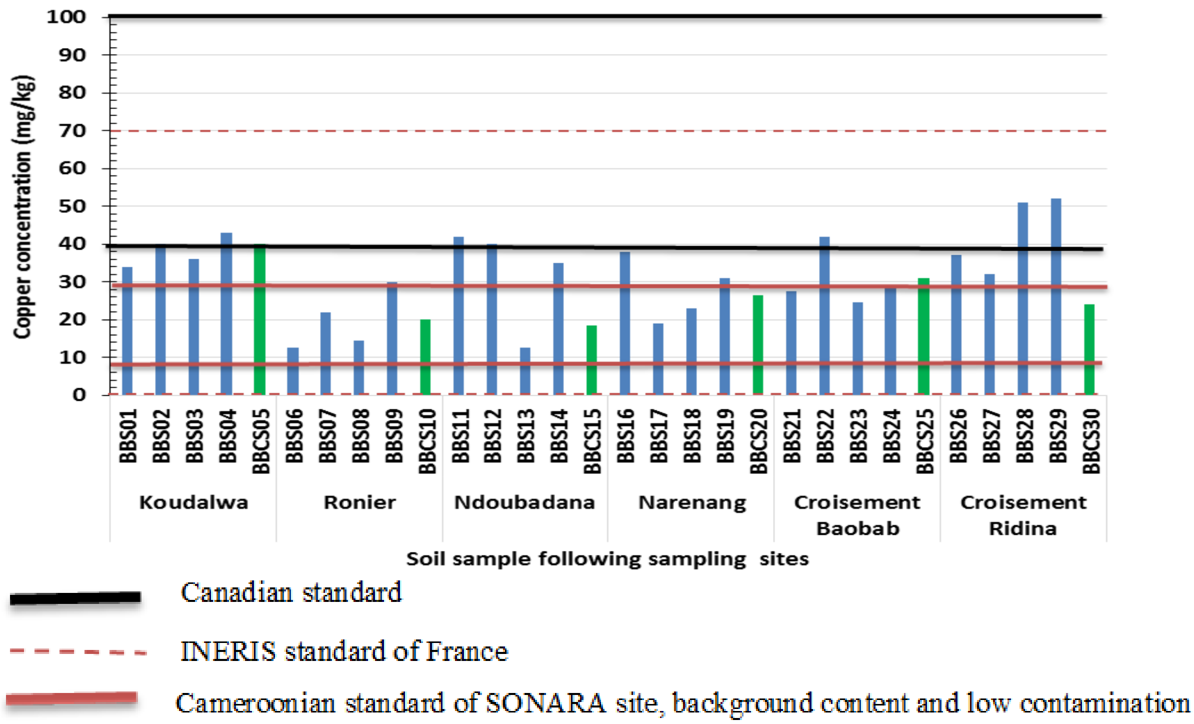

Figure 7. Copper concentration in the soil of oil fields and facilities.
BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

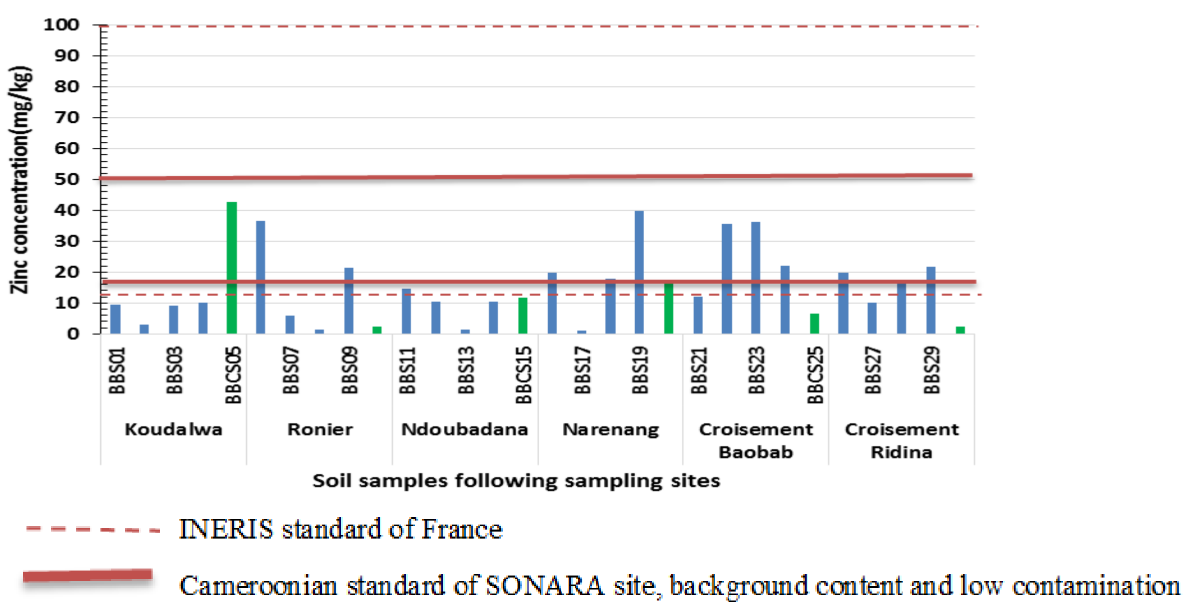

Figure 8. Zinc concentration in the soil of oil fields and facilities.
BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

sample BBCS05 of the same locality (Figure 8). In Ronier locality, zinc content of the control sample BBCS10 is lower than that of BBS06, BBS07 and BBS09 and higher than that of BBS08. For samples from the locality of Ndoubadana, zinc content of the control sample BBCS15 is below that of sample BBS11 and higher than that of samples BBS12, BBS13 and BBS14. Concerning the locality of Narenang, zinc concentrations of samples

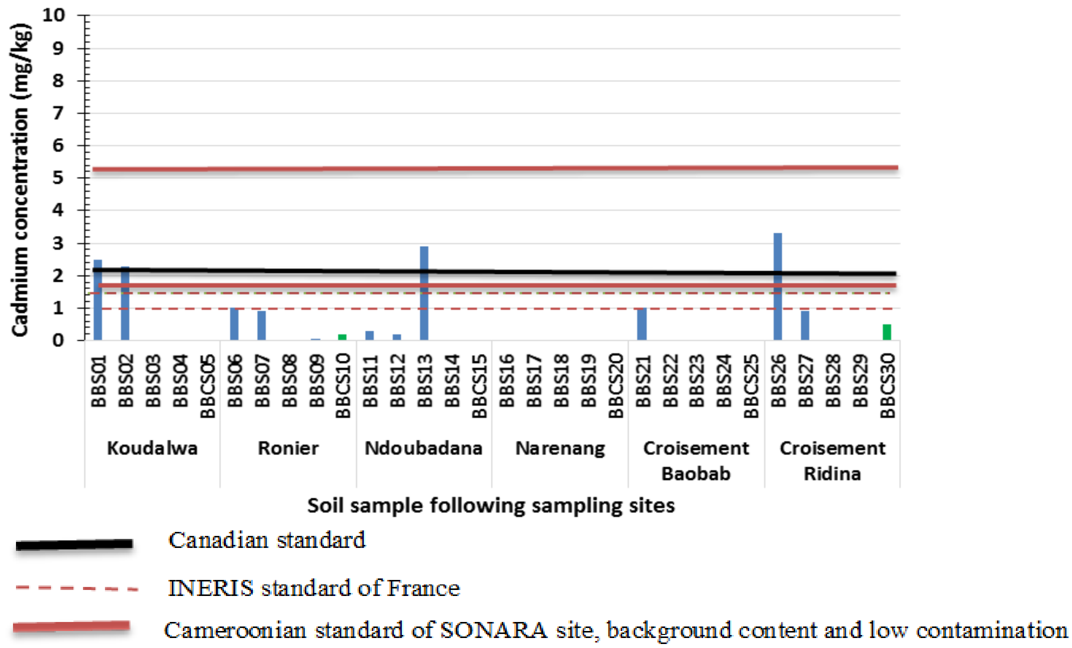

Figure 9. Cadmium concentration in the soil of oil field and facility sites.
BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

BBS16, BBS18 and BBS19 are higher than that of sample BBS17 and lower than the zinc content of control sample BBCS20. The zinc content of all samples at Croisement Baobab and Croisement Ridina are lower than that of control samples in these localities (BBS25 and BBS30 respectively) (Figure 8).

Cadmium

Cadmium detected from soil samples gives concentrations values that vary from 0 to 3.3mg/Kg with an average of 0.535mg/Kg and a coefficient of variation of 1.77, indicating strong presence of cadmium in soil of these environments. The high concentration values observed from samples BBS01, BBS02 of Koudalwa locality, BBS13 from the locality of Ndoubadana and BBS26 in Croisement Ridina, exceed the acceptable thresholds values of INERIS standards of France (0.2 - 0.7 mg/kg) and the low level of contamination of Cameroonian standards of SONARA site (1-2mg/kg) (Figure 9). These values of concentration are in the average contamination range of Cameroonian standards of SONARA site (>2 -≤5 mg/Kg).

Other chemical elements

The determination of the concentration of other elements such as Sulphur and Total Nitrogen samples has no significant results:

Sulphur

Sulphur content value of all samples in Bongor basin varies from 0.9 to 7mg/Kg; the average value is 4.26mg/Kg with a coefficient of variation of 0.42; that is an indicator of sulphur detection in soil of these localities. All of these values are very low and below the acceptable thresholds of the Canadian (400-2000mg/kg) and Cameroonian (60-120 mg/kg) (Figure 10) standards. Comparing the control samples to other samples from each locality, control sample BBCS05 in Koudalwa locality has higher sulphur content than all samples in that locality. In Ronier locality, the control sample has higher sulphur concentration than BBS07 in that locality. The control sample BBCS 15 has higher sulphur content than BBS11, BBS12 and BBS13 from Ndoubadana. The sulphur concentration of the control sample BBCS20 from Narenang is slightly above that of BBS17 and BBS19 of the same locality (Figure10). From the field observations, oil facilities have high standing ground to avoid stagnant water near theses installations therefore; drained oil spill and leakages by rain might lead to accumulation of oil content far from installations, where control samples are taken and making some of them to have high sulphur content.

Total nitrogen

Total nitrogen concentrations range from 0 to 56 mg/kg, the average value is 10.85mg/Kg, with a coefficient of

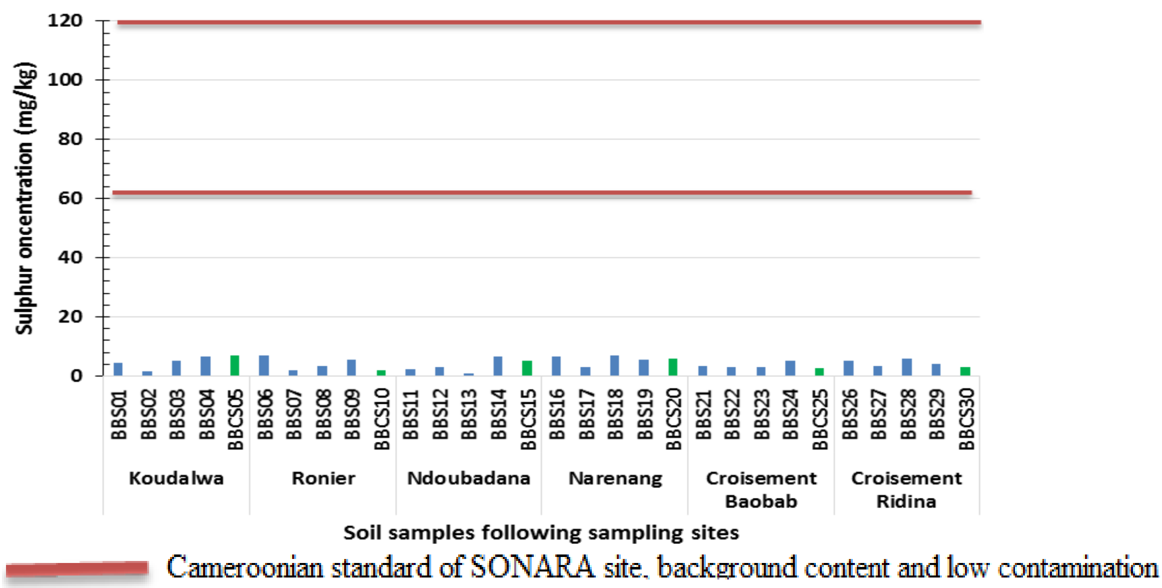

Figure 10. Concentration of sulphur in the soil of oil fields and facilities. BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

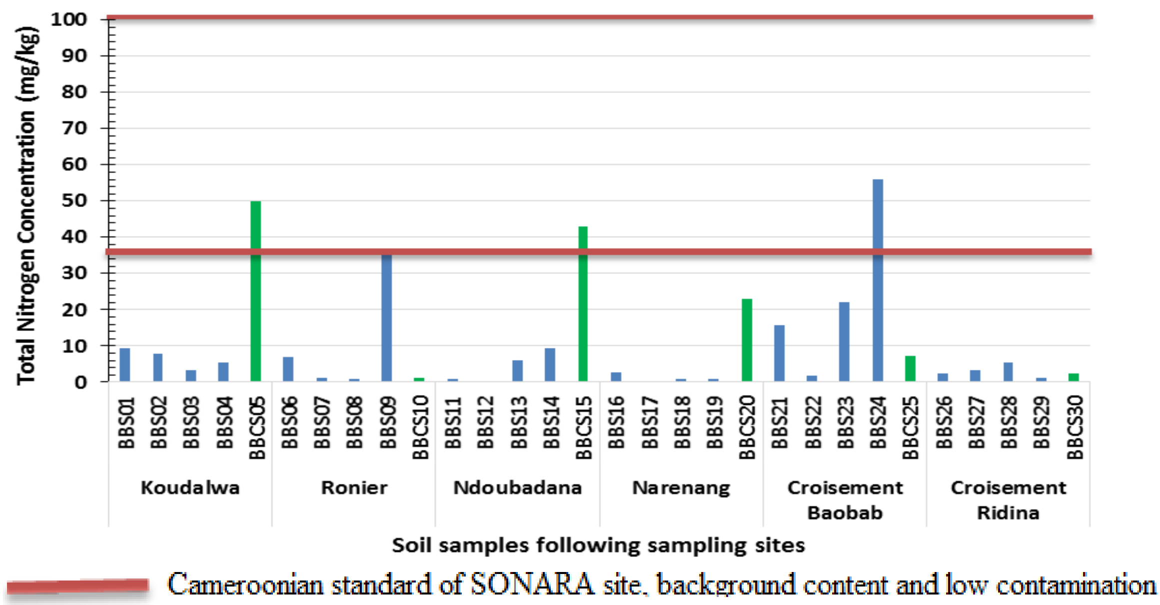

Figure 11. Total Nitrogen Concentration in the soil of oilfield and facility sites. BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

1.42. This indicates remarkable presence of nitrogen in the soil of these localities. All concentrations are below and within the Cameroonian standards of SONARA site (40-100 mg/kg). Control samples from the localities of Koudalwa (BBCS5), Ndoubadana (BBCS15) and Narenang (BBCS20) have higher nitrogen levels than other samples from these environments respectively. This low nitrogen value is as a result of oil content found in oil sites of Bongor basin with weight percentage of 0.26 W% (Anonymous 4, 2007). In addition, infiltration of nitrogen makes its content in soil relatively low in these areas. The control sample from the locality of Ronier has higher nitrogen content than that of BBS06 and BBS09 (Figure 11) sample. The control sample from Croisement Baobab is higher than that of BBS22. At Croisement Ridina, the control sample is slightly higher and higher

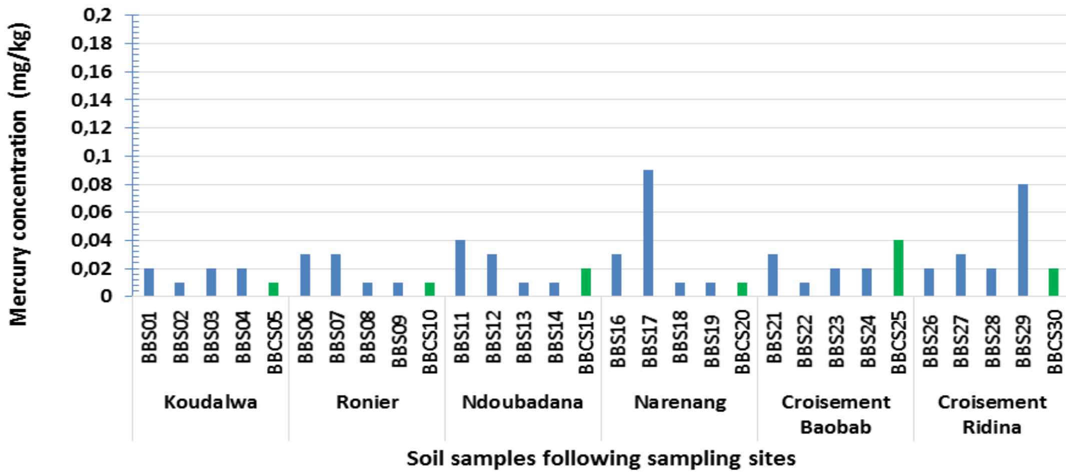

Figure 12. Mercury concentration in the soil of field sites and oil facility. BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

than that of BBS26 and BBS29 respectively (Figure 11).

Toxic chemical elements

Mercury

The value of concentration ranges from 0.01 to 0.09 mg/Kg with the average value of 0.024 mg/Kg with the coefficient of variation of 0.79 which is underlining the presence of mercury in all soil samples from the different localities of Bongor oil zone. Individual mercury concentrations of samples are low and are within the concentration range value of the acceptable threshold of the INERIS standards of France (0.02-0.10 mg/Kg) and below the Canadian standard (0.2 - 10 mg/Kg) and Cameroonian standards of the SONARA site (0.5 - 2 mg/Kg).

The control sample from Koudalwa locality has the same concentration value as that of sample BBS02. At Ronier, mercury concentration of the control sample has similar value to that of samples BBS08 and BBS09 (Figure 12). Mercury content of the control sample from Ndoubadana locality is higher compared to that of samples BBS11 and BBS12. In Narenang locality the control sample has the same concentration value as that of samples BBS18 and BBS19. In Croisement Baobab locality, the control sample has higher mercury content than that of other samples from the same locality. At Croisement Ridina control sample has the same concentration value as that of the BBS26 and BBS28 (Figure 12) samples.

Phenol

Analysis of soil samples for the determination of phenol concentration gives values that vary from 6.5 to 79mg/kg with an average of 26.23mg/kg and a coefficient of variation of 0.62, indicating strong presence of phenol in soil of these different localities. All samples have very high sample concentration values in comparison to Canadian Class A and B (0.1 - 1mg/kg) and Cameroonian SONARA site standards (0.2 -1mg/kg); however they are below the INERIS standard threshold (500mg/kg). Considering Canadian standards Class C (10mg/kg), one could observe low levels of concentration from samples BBS07and BBCS10 of Ronier locality and BBS17 of Narenang.

The control sample from the locality of Koudalwa is greater than the concentration of all samples from the same locality. One can also observe higher content of phenol in the control sample of Ronier locality than that of sample BBS07 (Figure 13). It is equally shown that control sample from the locality of Ndoubadana is higher than that of BBS13 sample. The control sample from Narenang and Croisement Ridina has higher phenol concentration than that of samples BBS17 and BBS27. We have higher phenol content of control sample from Croisement Baobab locality than that of samples BBS21 and BBS24 (Figure 13). This higher content of phenol in control samples is because of climatic conditions as mentioned earlier.

Total hydrocarbons

The concentration of total hydrocarbon of samples from different localities ranges from 2.4 to 22.6mg/kg, with an average value of 8.75mg/kg and a coefficient of variation of 0.51 expressing the presence of hydrocarbons in soil of these localities. We have low concentration values and are far below Canadian standards (300, 700 and 3500 mg/kg), INERIS standard of France (500 mg/kg) and

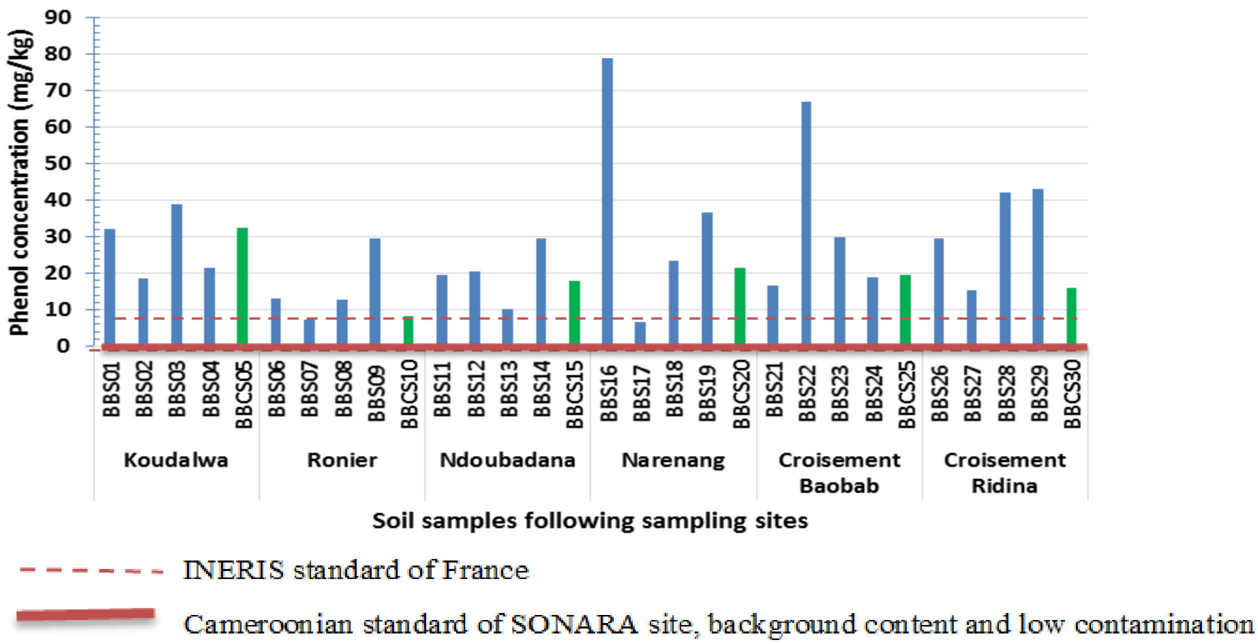

Figure 13. Concentration of Phenol in the soil of field sites and oil facility.
BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

Cameroonians standard of SONARA site (500 - 1000 mg/kg).It is observed that concentration of control sample in Koudalwa locality has the same value with sample BBS01 and higher than that of samples BBS02 and BBS4. In Ndoubadana and Narenang localities, control samples have higher total hydrocarbon content than that of samples BBS13 and BBS17, respectively. This phenomenon is in relation to the morphology of the zone (humidity of the soil) which appears to have an important role in the absorption of contaminants (oil product) by the sediments along water drainage (from rainfall), to different parts of the fields. It is also observed that, control sample of Croisement Baobab locality has the same concentration value with sample BBS21 (Figure 14).

DISCUSSION

Analysis of soil samples from Bongor oil sites for the determination of pollutants revealed the nature of contaminants that could disperse into surface water by runoff, alteration, erosion and then seep into groundwater.

The determination of the Nickel content gives high values of concentrations above the Cameroonian standard of SONARA site. High concentrations are observed from samples BBS03 (38.5 mg/Kg) of Koudalwa locality, BBS06 (102 mg/Kg) of Ronier locality, BBS16 (54 mg/Kg) of Narenang, BBS26 (50 mg/Kg) and BBS29 (39.5 mg/Kg) of Croisement Ridina locality. This soil contaminant comes from the nature of hydrocarbons, found in Bongor basin, which has 10g/g mass of constituents (Anonymous 4, 2007). These results obtained from Bongor Basin correspond with the one found in Bayelsa State, Nigeria, where the average nickel concentration (205 mg/kg) is above the permitted level (Okoro et al., 2020). Nickel is a metal heavily absorbed by soils but relatively mobile especially on acidic soils (Baize, 2000), so it is poorly accumulated by living organisms and seeps into groundwater (Bubb And Lester, 1996; Efsun and OlcayHüseyin 2015).

Considering Copper content of the soil samples from different localities, 16 out of 30 samples have high copper content which are above Cameroonian standards of SONARA site. These results are in line with the work carried out on sediments at Saint-François and Massawippi rivers banks in south-central of Quebec, which revealed higher levels of contamination with high concentrations of unusual copper according to the MDDEP (2004). High concentrations of copper observed in Absheron oil fields are above the accepted permitted levels of Azerbaijan Republic (Khalilova, 2015). These high concentrations of Cu could be in particular due to its use as corrosion inhibitors (Economides et al., 1989) and in electrical and electronic equipment (electromagnets, relays, distribution bars, switches, circuit boards), cathode-ray tubes, lightning rods or electronic gadgets abandoned at oil sites; which is also the case in Bongor basin oil sites. Similar results are presented with extremely high concentrations of copper at electronic waste sites in China (Zhang et al., 2012).

According to Frischer (2013), copper in the soil has

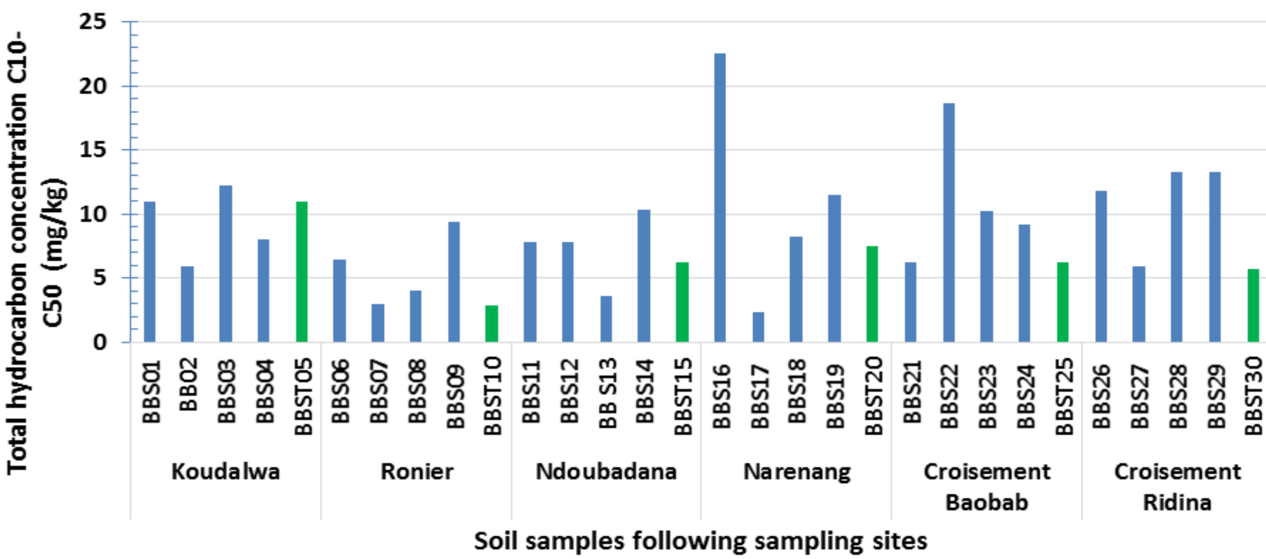

Figure 14. Concentration of Hydrocarbons in the soil of field sites and oil installation.
BBS – Bongor Basin Soil sample from oil site or fields, BBCS- Bongor Basin Control Soil sample.

significant adsorption on iron oxides, manganese, clay and organic matter with low mobility. These insoluble significant adsorption on iron oxides, manganese, clay and organic matter with low mobility. These insoluble compounds are present in the form of immobile precipitates in soils; they are also likely to change in nature, depending on physicochemical conditions and the presence of suspended particles. According to Maleckiet al. (1982), copper toxicity depends on their speciation, and copper is more bioavailable in soils that are low in organic matter (Streit 1984; Donkin and Dusenbery, 1993).

In the case of cadmium content of the samples, 8 out of 30 samples have concentrations above the acceptable threshold of INERIS standards of France and 4 out of 30 samples above the Cameroonian standard of SONARA site. The results from the analyses showed concentrations as high as of those carried out on industrial and anthropogenic discharge deposits in the two ports city of Sfax and Gabès (Chouba and Mzoughi-aguir, 2006) with respectively 10.42 mg/kg and 26.34 mg/kg. In the study area, this high concentration is from the nature of hydrocarbons found in Bongor Basin, which have 4.9 µg/g concentration of cadmium (Anonymous 4, 2007). The high content of Cadmum found might be from its mobility and its ability to accumulate in the upper horizons of the soil rich in organic matter (Onweremadu and Duruigbo, 2007). It could also come from alloys used for electrical conductors, fuses, coatings as anti-corrosion properties, which is unalterable in the air.

Analysis of samples from oil sites in the various localities of Bongor basin indicates high phenol concentrations that range from 6.5 to 79 mg/Kg. These sample concentration values are very high compared to Canadian standard Class A and B (0.1 -1mg/kg) and Cameroonian standards of SONARA site (0.2 – 1mg/kg), signifying high phenol soil contamination of these different localities in Bongor basin. Similar on-site studies of abandoned refinery in China have shown high concentrations of phenol compounds ranging from 0.01 to 232.96 mg/kg (Pei et al., 2012). Phenol was the main pollutant of oil shale semi-coke discharge leachates that contaminate surrounding soils in northeastern Estonia (Kahru et al., 2002). High concentration of phenol could be due to various surface water activities (treatment of large quantity of water produced from oil wells in Bongor basin), well stimulations and sand control techniques carried out in wells (Motta, 1993; API, 1991; Economides et al., 1989). Phenol is easily absorbed by clays once on the ground (Zhang and Spark, 1993). According to Howard (1989), phenol is mobile in the soil, soluble in water and moderately volatile; it can be easily leached from soils and contaminate groundwater by giving poor or bad water taste.

Detected parameters such as Chrome, mercury, sulphur, total nitrogen and total hydrocarbons from the soil samples analyzed have low levels of concentration in different localities during this study. This scenario is that concentrations often depend on events associated with spills and environmental conditions, hydrological conditions, e.g. sedimentary and climatic conditions that favor its migration deep beneath the subsurface (Nikanorov and Stradomskaya, 2003).The presence of mercury was detected from soil samples in Bongor with low concentration (giving a maximum value of 9 g/l) compared to the low Cameroonian standard contamination threshold of SONANRA site. Even at low concentrations, mercury is a major danger to microorganisms and other biota; these levels of concentrations might have adverse effects on the environment even at 5g/l (Boening, 1999, Orctet al., 2006). This low concentration of Hg in most soil samples taken from Bongor basin is the fact that, Hg easily evaporates as organo-mercury due to changes in climatic conditions (temperature) of the environment (Environmental Health - Safety Manual, 2000). The main sources of anthropogenic emissions of mercury, including other heavy metals (cadmium, lead, etc.) are thermal power plants (gas turbines from oil sites) and waste combustion industries (Vermillion et al., 2005).

The pH determination of soil samples from the Bongor basin gives an average value of 7.39 and is within the range of arable soil pH value, a maximum value of 8.85 in the alkaline soil range and a minimum of 4.8 that is in the acid range (Figure 7). The minimum value, which is within acid range could come from the nature of matrix stimulation fluids with the use of acids such as hydrochloric and hydrofluoric acid (Motta, 1993). And values within the alkaline range could be related to drilling mud and completion fluids mixed with sodium chloride, ammonium chloride fluids additives and as well as formation water produced with hydrocarbons to the surface (European Commission, 2019). Acid pH influences the mobility of heavy metals in soil; an alkaline pH limits the passage of heavy metals from solid phase to liquid phase in soil and then its transfer to plants (Thornton, 1996).

Compared to acid/base reactions, with a reduced pH, this enables most chemical elements transport, due to increase of their solubility; with acidic soil, this facilitates heavy metal elements dissolution contained in core mineral (Baize, 2000), therefore the transfer of these elements into deep aquifer formations.

Conclusion

Results from laboratory analyses revealed the nature of the pollutants in soil samples from various oil sites in Bongor basin, such as Ni, Cu, Cd, Hg and Phenol, which are indicators of soil contamination in these environments. Their values of concentration are 102, 52, 3.3, 0.09 and 79mg/kg respectively and above Cameroonian standard of SONARA site, Canadian standard and INERIS standard of France (500mg/kg). The determination of pH made it possible to assess the acid/base reactions of these environments and its influence on the mobility of metals in the soil. For this purpose, soil samples taken from Bongor oil sites have a maximum pH value of 8.85 that is within the alkaline soil range, with the average pH value of 7.39 in the range of the pH value of the arable soil, and a minimum of 4.8 within the range of acid soil content.

An alkaline pH limits the passage of heavy metals from the solid phase to liquid phase in soil, and a reduced pH favours the transport of most chemical elements to deep aquifer formations. The presence of these pollutants in soil taken from oil sites in Bongor basin whether low or high in concentration could be persistent in the environment and cause serious risks to environment and particularly to qualitative nature of ground water resources.

Recommendations

Further assessment of water pollution (pollutants) from surface waters (rivers and streams), traditional wells and human-motorized water boreholes in Bongor basins is needed. In addition, geochemical modelling studies of mechanisms governing mobility, infiltration or passage of pollutants from the soil to surface water and then groundwater in Bongor basin oil sites are very important in order to see the level of contaminations in the whole area. Results obtained will be valuable contributions for environmental protection measures and decisions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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