UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

(3/2021) 36

PUBLISHER UWM
OLSZTYN 2021

EDITORIAL BOARD
Małgorzata Woźniak (Editor-in-chief), Małgorzata Jankun-Woźnicka (Deputy Editor-in-chief), Anna Źróbek-Sokolnik (Biology and Forestry), Mirosław Wyszkowski (Agriculture), Katarzyna Majewska (Food Science), Dorota Fopp-Bayat (Fishery), Józef Szarek (Veterinary Science), Iwona Gołaś, Jolanta Grochowska (Environmental Protection), Andrzej Gugołek (Animal Breeding and Husbandry), Vaclav Matoušek (Animal Science, Czech Republic), Juraj Mlynek (Animal Behavior, Slovak Republik), Grażyna Furgała-Selezniow (Humans and Environment)

Executive editor
Agnieszka Orłowska-Rachwał

The Polish Journal of Natural Sciences is indexed and abstracted in Biological Abstracts and Biosis Previews

The print edition is the primary version of the Journal

The Journal is also available in electronic form on the websites
http://www.uwm.edu.pl/polish-journal/ (home page)
http://wydawnictwo.uwm.edu.pl (subpage *Czytelnia*)

PL ISSN 1643-9953

© Copyright by Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego
Olsztyn 2021

PUBLISHER UWM OLSZTYN

Address
ul. Jana Heweliusza14
10-718 Olsztyn-Kortowo, Poland
tel.: +48 89 523-36-61
fax: +48 89 523-34-38
e-mail: wydawca@uwm.edu.pl

Ark. wyd. 11,4, ark. druk. 9,25, nakład 75 egz.
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 30

TABLE OF CONTENTS

Agriculture

Biology and Forestry

Environment Protection

Fisheries

Humans and Environment

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 215–228, Y. 2021

EFFECT OF DEPTHS OF TILLAGE ON THE PERFORMANCES OF OPEN-POLLINATED YELLOW MAIZE (*ZEA MAYS*) VARIETIES

***Wasiu Agunbiade Lamidi*[1], *Mosobalaje Abdulsalam Murtadha*[2], *Michael Segun Afolabi*[3], *Adedamola Emmanuel Olawumi*[4]**

[1] ORCID: 0000-0003-4147-9292
[2] ORCID: 0000-0002-3196-2582
[3] ORCID: 0000-0002-2698-9828
[1] Department of Agricultural Engineering, Faculty of Engineering, College of Science, Engineering and Technology
Osun State University, Osogbo, Nigeria
[2–4] Department of Agronomy, Faculty of Agricultural Production & Management, College of Agriculture, Ejigbo Campus
Osun State University, Osogbo, Nigeria

Key words: tillage depth, maize, leaves number, plant heights, silking, yield component.

Abstract

The study investigated the depths of tillage on the yield of open-pollinated maize (*Zea mays*) varieties. Eighteen compartmental plots of 400 m^2 at 4 m × 10 m; 2 m apart, each comprising 2 depths of tillage (0–15 cm and 0–30 cm), 3 open-pollinated varieties (OPV 1, OPV 2, OPV 3), each replicated thrice (2 × 3 × 3 factorial design) were experimented. Data collected were days to emergence of seeds, plant height, the number of leaves/plant, days to 50% anthesis (DAT), days to 50% silking (DAS), Anthesis-Silking Interval (ASI), Leaf Area Index (LAI), grain yield (GY)/ha. There were no statistical differences for the number and length of leaves and DAT, there were statistical differences for plant heights, DAS, LAI, stem girth and ASI. OPV 3 had the highest number of leaves, plant height, stem girth, while OPV 2 had the highest mean value for LAI and DAT. Depth 0–15 cm was adjudged the best in the study.

Introduction

Maize production in Nigeria over the years has been decreasing, as against the increase in the country's population, this necessitated the government to import maize for both man and animals' consumption (MOJEED 2020). This decrease in supply and production may not be unconnected with environmental and soil factors, crop management techniques and some government policies.

Address: Wasiu Agunbiade Lamidi, College of Science, Engineering and Technology, Osun State University, Osogbo, Nigeria, phone: +2348135860977; +2348062085175, e-mail: wasiu.agunbiade@uniosun.edu.ng

Compared to the world, Nigeria has not been doing well in maize production rate as compared with the global figures. As of 2019, maize production in the United States of America was 347,047,570 tonnes, China 260,957,662 tonnes, Brazil 101,138,617 tonnes and Nigeria had 110,000,000 tonnes (WITS 2021, FAOSTAT 2020, NUEWEB. 2012). Factors responsible for this low production in Nigeria could not only be because of global climate change, but also management, non-usage of soil enhanced materials, nature of seeds and other production strategies that may be peculiar to African states. The question now is, what could have happened to Nigeria that made her maize production rate dwindle over time? Likely factors from locality may also be the depth of tillage, depth of sowing, planting of varieties that were not adaptable to local climate, management efforts of the husbandry and others (PITTELKOW et al. 2015, OLADEJO and ADETUNJI 2012).

The ultimate goal of agricultural production is high yield which has a direct relation with measures of tillage. When tillage is improper, it can cause severe land degradation, and low yields (DONI et al. 2017). Many scholars who have dealt with tillage have also considered the negative effects of tillage on the plant, i.e. its ability to increase the rate of water runoff; the over-tilling effect on soil structure; decrease in soil-water infiltration rate, dislodging the cohesiveness of the soil particles and inducing erosion; reduction in soil organic matter and destruction of soil aggregates (HORTON 2019, TAMBURINI et al. 2016, ANDERSON and D'SOUZA 2014). Similarly, some reports demonstrated that conservation tillage (0–20cm) can improve soil fertility (WANG et al. 2019), maintain maize yield (SHAO and BAUMGARTL 2016), and guarantee increased production (REN et al. 2016). Deep tillage (above 25 cm) according to MRABET (2011) enhanced significantly the number of grains per spike, 1000 grain weight of maize and the final grain yield by breaking hard bottoms (MA et al. 2015), improving soil pore conditions, and increase permeability. These characteristics are needed for the distribution of plant roots in deep soils and the proper use of nutrients and water therein (SUN et al. 2013).

As important as varieties and cultivars are to crops' management and performances on the field, there are other factors aside from tillage practices that may be responsible for high crops' productivity on the field. Conservation tillage reduces damages of young plants from blowing sand (sandblasting) because it decreases the splash of soil onto plant parts during rainfall and it suppresses weeds' growth. As tillage does all these to crops, maize, being a fibrous root plant may not necessarily need deeper depth than 30 cm for its good performance. However, the effect of depth shallower than 30 cm on maize growth needs thorough investigation to check the nutrients' availability for plant growth, rate of yields obtained at varying depths of tillage and studying the correlation between depths of tillage and performances of different open-pollinated varieties of maize.

Materials and Methods

Experimental site

The field experiment was conducted at the Teaching and Research Farm of the College of Agriculture, Osun State University, (7.8717N 4.3067E) Ejigbo campus from April to August 2020. The climate was rain forest with bimodal rainfall between 1,158–1,250 mm per annum. The temperature regime is usually high all year round with a mean of 28–33°C, relative humidity of about 85%, except during the dry season with the sunshine of 5.1%. The climate of the area is characterized by pronounced wet and dry seasons, moderate temperatures in the wet season and relatively higher temperatures with moderate humidity during the dry season.

Land preparation, experimental layout and design

The experimental field was cleared manually to be sure of effective mechanical cultivation and to have desired soil depths of 0–15 cm and 0–30 cm. The dimension of the land was 73 m × 100 m. The land was divided into eighteen compartmental plots of 400 m^2 each comprising 2 depths of tillage (0–15 cm and 0–30 cm), 3 open-pollinated varieties (OPV 1, OPV 2, OPV 3), all at 3 replicates each to make 2 × 3 × 3 factorial design. The tillage treatments were laid out in randomized complete block design and replicated three times, Figure 1. Each plot measured 4 m × 10 m at 3 rows for each treatment was 4 m distance from the next (for passage of power tiller). Another headland of 10 m spacing from the entire areas of land, between experimental blocks was provided for power tiller passage and implement hitching process. The treatment consisted of the following tillage practices: ploughing as the first phase, ploughing followed by harrowing as the second phase. Both phases were within the procedural depths of tillage in the experiment and within two weeks intervals. At both stages, the ploughing depth and harrowing depth were within either the 0–15 cm or 0–30 cm tillage depths designed for the experiment. In all, the range of sowing depth of 6–10 cm was chosen as the depth of sowing in the experiment to have the highest plant height (BERHANU et al. 2016).

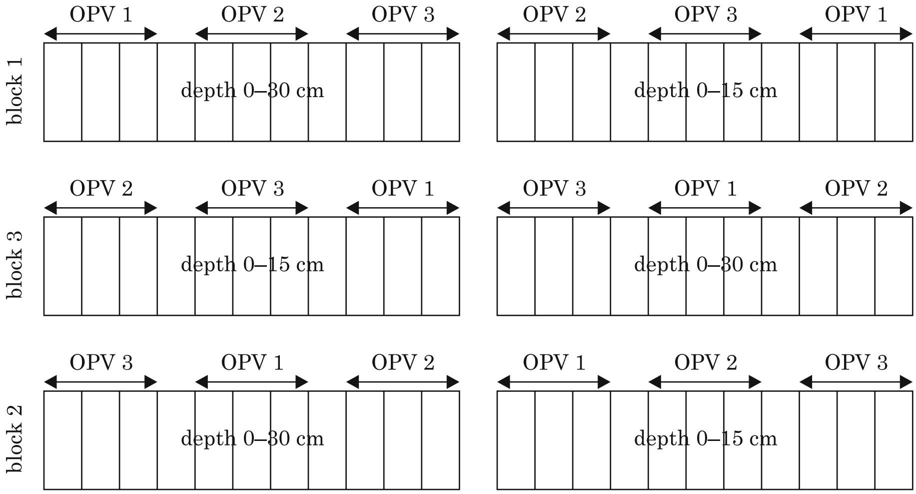


Fig. 1. Experimental layout of randomized compartmental plots

Configuration of disc plough to a depth of tillage desired

Power tiller, model name: Kubota PEM140 DI, 4-cycles, 10HP, 2400 rpm, made by Vikas Motor Co, India was used because it was easy to control the speed of the mechanical cultivation, and to be able to maintain the depths required according to the experimental procedures. Mounted disc plough, 3 furrows (Devta, made in India) were marked in the cylindrical jig, regarding their axes to synchronise with the precise 15 cm and 30 cm depths' marks required. These points were marked with yellow and red indelible paints at different points, yellow from the disc centre edge up on plough toward the disc circumference to make either 15 cm or 30 cm from the circumference where the 15 cm or 30 cm was painted red. There were two concentric circles in each disc, inner painted yellow, outer painted red at 15 cm or 30 cm, Figures 2a, 2b. The red point was always above the desired depth while the yellow was always the mark of the precise desired depth of tillage. The red marked line is on the outer disc to the edge. This was to minimize the influence of the roll angle during tillage operations along the elevation of the non-tillage road surface (KIM et al. 2020).

The power tiller was made to maintain a uniform speed of 5 km h^{-1} throughout the operation in each case as the plough was adjusted for the required depths in each situation. The low speed of the power tiller allowed the marked yellow-coloured spots and frame to be visible throughout the operation for both depths in all cases.

Soil samples were taken at a depth of 0–15 cm and 0–30 cm using random sampling techniques and analysed at the laboratory of the College of Agriculture of the Osun State University. The samples were mixed to form composites, air-dried and sieved using a 2 mm sieve.

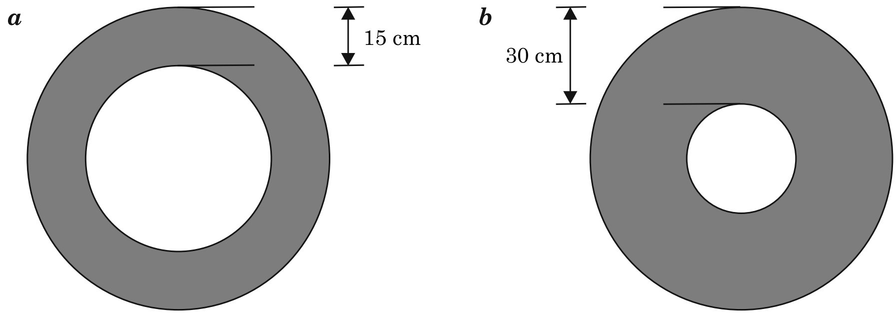


Fig. 2. Skech of plough at : *a* – 15 cm; *b* – 30 cm marked depth

Determination of growth parameters

The agronomic parameters collected include days to emergence of seeds, plant height (cm), number of leaves/plant, days to anthesis (DAT), days to silking (DAS), stem girth, Anthesis-Silking Interval (ASI), Leaf Area Index (LAI) and grain yield (GY) t ha^{-1} (EDMEADES et al. 2000).

Plant height [cm]: the heights of 10 randomly tagged plants were measured every two weeks from the ground level to the tip of the terminal leaf with the aid of a meter rule.

Number of leaves/plant: at least 20 representative plants were counted on after the milk stage

Days to anthesis (DAT): the plants in the three middle rows in each plot were used to estimate days to 50% tasseling. That is the number of days from sowing to when 50% of the plants have shed pollen.

Days to silking (DAS): the plants in the three middle rows in each plot were used to estimate days to 50% silking. That is the number of days from sowing to when silks have emerged on 50% of the plants

Stem girth: the girth (diameter) of the plant stem was taken weekly at the base of each plant about 5 cm above ground level with the aid of a vernier callipers. Ten (10) plants were randomly tagged from the fourth week to the tenth week for the girth data.

Anthesis-silking interval (ASI): this is measured as the differences between days to tasseling and days to silking.

Leaf area index (LAI): A quadrat of 2 m^2 area was selected randomly at 8 weeks after sowing where optimum plant growth was achieved from each plot. The measurement of length and width at the broadcast point of each leaf in the quadrat was then taken. Each leaf area designated as A was estimated by the formula:

$$A = L \cdot B \cdot 0.75$$

where:

L – the length of the leaf

B – the maximum width of the leaf [cm]

0.75 – the correction factor.

Grain yield (GY) t ha^{-1}. Grain Yield (kg ha^{-1} = (Ear weight (kg)/area in m^2).

Fertiliser application was not a factor in the research since an equal measured quantity of NPK 15: 15: 15 was used for the same plant in all the fields.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA), the means were separated using Duncan Multiple Range Test at a 5% level of significance.

Results

Maize yield components' performances at different tillage depths

The mean performance of three maize varieties at different tillage depths is represented in Table 1. There were no statistical differences ($p \leq 0.05$) in the values obtained for the days to 50% silking and days to 50% anthesis, but there were statistical differences for the length of leaves, LAI, stem girth and Anthesis-silking interval. OPV 3 had the highest number of leaves (11.33), Figure 3; plant height (144.78), stem girth 1.72 for 0–30 cm depth, Table 2 while OPV 2 had the highest mean value for leaf area index (8.97) and days to 50% anthesis (51.72).

Table 1

Mean values showing performances of three maize varieties

Varieties	LL [cm]	LAI [cm^2]	SG [cm]	DAT	DAS	ASI
OPV 1	87.75±0.71^b	8.90±0.37^a	1.54±0.08^b	51.39±0.24^a	56.17±0.41^a	4.78±0.75^a
OPV 2	97.17±0.75^a	8.97±0.41^a	1.64±0.06^a	51.72±0.27^a	55.39±0.46^a	3.67±0.11^{ab}
OPV 3	92.05±0.49^{ab}	8.68±0.13^b	1.65±0.07^a	51.56±0.48^a	55.44±0.43^a	3.89±0.15^b

^{ab} means on the same column with different superscripts are significantly different ($P < 0.05$). LL – length of leaves; LAI – leaf area index; SG – stem girth; DAT – days to 50% anthesis; DAS – days to 50% silking; ASI – anthesis-silking interval

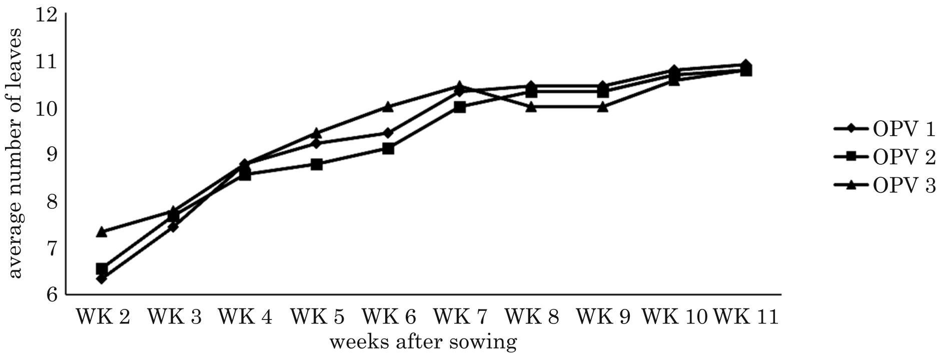


Fig. 3. Average weekly number of leaves per plant from OPVs at tillage depth of 0–15 cm

Table 2

Mean values showing different tillage depths on the performances of maize

Depth of tillage	LL [cm]	LAI [cm^2]	SG [cm]	DAT	DAS	ASI
0–15 cm	90.67±6.22^b	8.87±0.75^a	1.50±0.51^b	50.77±0.13^b	54.85±6.74^b	4.07±0.18^b
0–30 cm	93.98±5.49^a	8.82±0.81^b	1.72±0.14^a	52.33±0.15^a	56.48±7.10^a	4.14±0.21^a

^{ab} means on the same column with different superscripts are significantly different (P < 0.05). LL – length of leaves; LAI – leaf area index; SG – stem girth; DAT – days to 50% anthesis; DAS – days to 50% silking; ASI – anthesis-silking interval

The average number of leaves on selected marked maize stands among the replicates 1, 2, 3 and the varieties OPV 1, OPV 2 and OPV 3 are 9.41, 9.28 and 9.51 respectively for 0–15 cm depth and 9.96, 9.53 and 9.97 for 0–30 cm depth. In both depths, only OPV 1 and OPV 3 were statistically different from each other. From their standard deviation values depicted in Tables 1 and 2, they are closer to each other signifying lesser deviations in the values of the number of leaves. In Figures 3, 4, the number of leaves increased with an increase in number of weeks after sowing, as expected.

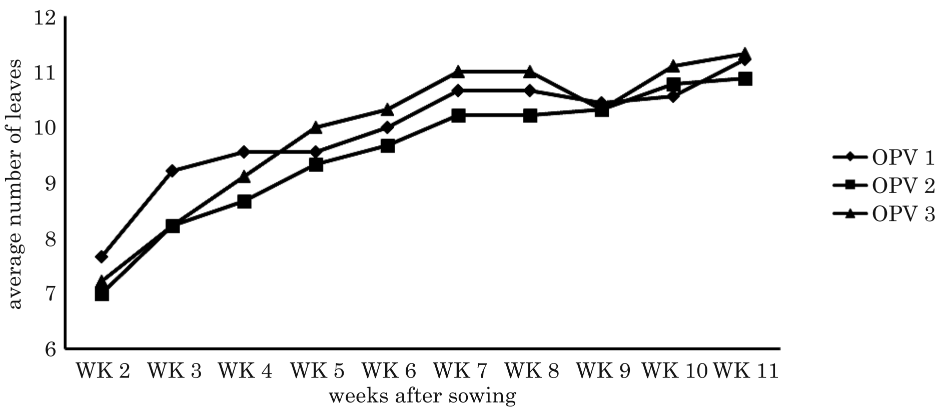


Fig. 4. Average weekly number of leaves per plant from OPVs at tillage depth of 0–30 cm

Also, there was constancy in the number of leaves starting from week 7, this happened in both depths, showing that healthy growth of tasseling has been initiated. However, the number of leaves were different for varieties OPV 1, OPV 2, OPV 3 at both depths of tillage.

On the mean values showing different tillage depths on the performance of the three maize varieties, Table 2 shows that the mean values of different tillage depths were statistically different for the length of leaves, leaf area index, stem girth, anthesis-silking interval and for all other yield components. The plant height, stem girth, days to 50% anthesis and days to 50% silking were all lower at 0–15 cm with 111.22, 1.50, 50.77 and 54.85 than corresponding values at 0–30 cm depth of tillage respectively, Tables 1, 2; Figures 5, 6.

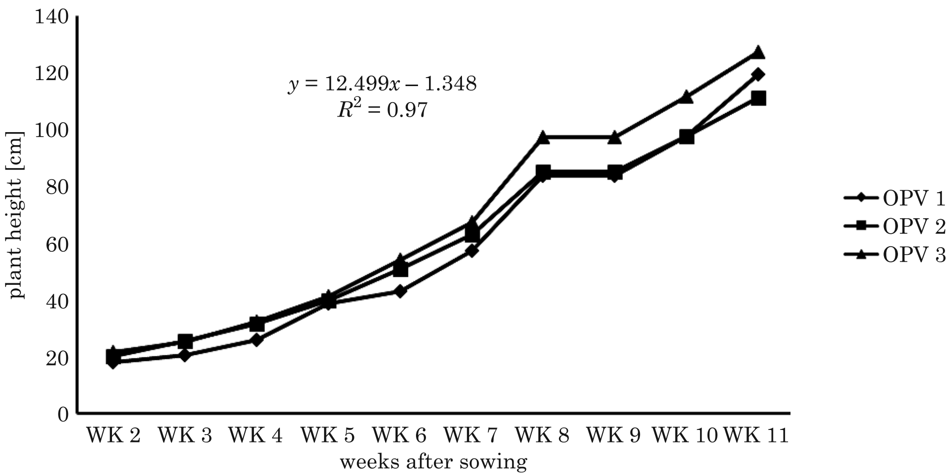


Fig. 5. Average heights of plants from OPVs at tillage depth of 0–15 cm

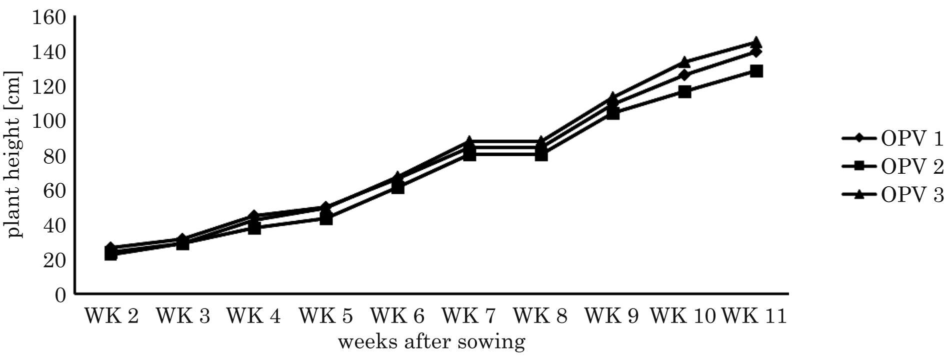


Fig. 6. Average heights of plants from OPVs at tillage depth of 0–30 cm

The yield of three maize varieties at different tillage depths

Table 3 shows the values of cob weights of the three post dehusking and pre dehusking maize varieties. It is of note that at both 0–15 cm and 0–30 cm depths of tillage, there were statistical differences among the mean values of the number of cobs and weights of cobs at the both before and after dehusking among different varieties OPV 1, OPV 2 and OPV 3. Similarly, there were statistical differences among the mean values of the harvested weights' recorded from cobs before and after dehusking among different varieties OPV 1, OPV 2 and OPV 3.

After dehusking cob weight was the highest at OPV 3 in 0–15 cm depth (8.67 and the lowest at OPV 2 (5.00). It is also worthy to note that varieties planted at 0–15 cm had the highest cob numbers and after dehusking cob weights than 0–30 cm depth. The three varieties have good morphological parameters that could have led to their moderate performances culminating in the highest yield of 9.90 t ha^{-1} recorded for the OPV 3 at 0–15 cm tillage depth. This was a 25.32% increase over the yield 7.90 t ha^{-1} recorded in the 0–30 cm depth of tillage.

Table 3

Cob weight yields of three maize varieties at different tillage depths

Tillage depth	Type	Variety	Mean	±SEM
0–15 cm	post-dehusking	OPV 1	6.77	0.34
		OPV 2	6.27	0.70
		OPV 3	8.67	1.70
	pre-dehusking	OPV 1	7.30	1.00
		OPV 2	7.33	2.03
		OPV 3	9.90	2.33
0–30 cm	post-dehusking	OPV 1	5.47	0.29
		OPV 2	5.00	0.58
		OPV 3	6.87	1.35
	pre-dehusking	OPV 1	5.83	1.15
		OPV 2	5.57	1.91
		OPV 3	7.90	2.23

Discussion

Yield components of different maize varieties at different depths of tillage

With OPV 3 having the highest number of leaves, plant height and stem girth, this may be as a result of genetic characteristics of the individual maize varieties. The findings of ENUJEKE (2013) agree with this, he reported that the variations in genetic constituents of the crops could lead to differences in growth indices of some crops as there could be physiological differences in their features. Values recorded for Anthesis-silking interval across all varieties indicated that they are modern and important for maize crop to be productive, for OPV 2 to have the value of anthesis-silking interval (3.67), although lower when compared to OPV 3 (3.89) which was 5.6% higher, both values could depict the fact that these two varieties have desirable genetic characteristics that can promote the much evidenced development in these yield components: number of leaves, plant height and stem girth in this open-pollinated variety provided they are adequately managed by the farmer. Also, these OPVs have the potentials of producing really high yields as evidenced in the values recorded especially for ASI across all the varieties. This is in line with the reports of ENUJEKE (2013) and MAGOROKOSHO et al. (2003). It can also be surmised that genetical variation for ASI may indicate differences in the relationship between other growth parameters (number of leaves, plant height, stem girth and LAI) and yield components (ASI, DAS, and DAT) and hence differences in partitioning of any could-be-formed of these growth parameters or yield components may assimilate to the crop's ear at flowering. It could also be attributed to the differences in yield components in maize cultivars to stomata conductance value and differences between genotypes in the partitioning of photosynthetic materials towards economic yield. (ENUJEKE 2013, MAGOROKOSHO et al. 2003, EDMEADES et al. 2000).

The highest number of leaves, plant height, leaf length and stem girth obtained for these maize varieties planted at the tillage depth of 0–30 cm agreed with the findings of ENUJEKE (2013) MRABET (2011) and MOLATUDI and MARIGA (2009). In contrast, WATO (2019), in his study reported that the shallower depth of planting for maize had the highest number of leaves, plant height, leaf length and stem girth than the deeper depth of planting. Although depths of planting are not the same as depths of tillage, though they are not independent of each other, therefore, the results of this experiment agreed with WATO (2019) findings on how depths of planting, depths of tillage affected the yield components in different maize varieties he experimented upon. Also, other factors like environmental parameters (not researched into in this study) may be responsible for differences in the yield parameters as revealed by these results, moreover, that depth of planting was within the depth of tillage 0–15 cm. The contrast on how shallower and deeper depths of planting affect performances of maize on the field may be a result of edaphic and environmental characteristics or genetic characteristics of the varieties used in this study. More investigations are needed to determine the effect of any of these factors on the yield components under tillage and planting.

Although 0–15 cm depth of tillage gave the highest yield of maize in the research, however WANG et al. (2019) reported that soil properties and crop yield improved when deep tillage of more than 25 cm was used in the North China Plain (which is a temperate area). This contrast may be so because of the differences between what temperate zone of the world may offer and what the tropical soil and environment may add to crops' growth and yield. In addition, tillage was also advantageous under water stress as the uptake of subsoil water and crop yield were increased. It could also be an observation related with the ability of tillage depth on the soil granular structure and also on the depth to which the root of the crop was restricted under shallow tillage and variations in the water and nutrient supply to the crop (PIAO et al. 2016).

Starting from week 7, there was apparent constancy in the number of leaves as more leaves were not added, this happened in both treatments. This shows healthy growth as tasseling should occur then. However, the number of leaves was different for varieties OPV 1, OPV 2, OPV 3 at both depths of tillage. This could be because of the differences in the depths of soil cut and the residual effects on the roots of maize which would allow their development in the soil to reach different depths due to factors like permeability, nutrients' ease of absorption and ease of soil aeration. This opinion agrees with PIAO et al. (2016).

The yield of different maize varieties at different depths of tillage

Maize varieties planted at 0–15 cm tillage depth had a better number of cobs than those planted at 0–30 cm, and out of the three varieties, at 0–15 cm OPV 1 had the highest mean number of cob while OPV 3 had the highest mean cob weight (9.90). These variations in yield may be the result of special genetic qualities possessed by the maize varieties. This opinion can be strengthened by the reports of SEID et al. (2013) and MOLATUDI and MARIGA (2009) who reported that some maize varieties have yield advantages over other maize hybrids due to their possession of special genetic qualities. These genetic qualities could have been therein in their cultivars and could have been intentional by the breeders or might have resulted from physiological processes within the seeds then or thereafter. The evident variations in yield of cob seen could also be related to tillage depth as 0–15 cm depth was better than 0–30 cm depth of tillage as shown in the 25.32% increase of OPV 3 in 0–15 cm depth over the yield, 7.90 t ha^{-1} recorded in the OPV 3 in 0–30 cm depth of tillage. The report corroborated WANG et al. (2019) and SHAO and BAUMGARTL (2016) who showed that conventional tillage increased maize yield.

Moreover, the statistical differences among the mean values of the harvested weights' recorded on cobs before and after dehusking among different varieties OPV 1, OPV 2 and OPV 3 could be due to some factors like variety differences, depths of tillage which could affect the development of roots and by extension affected shoot development in the maize as evidenced from the Figures 3–6 on the growth parameters namely the number of leaves and plants' heights. Also, these statistical differences among the yield components and growth parameters may be due to some other soil factors especially the soil biotic factors that were not taken into consideration as the experiment held them constant throughout for all the treatments in that period.

Conclusion

The study investigated the effect of different depths of tillage on the yield components of field tilled, sown with open-pollinated maize (*Zea mays* L.) varieties. Three varieties of open-pollinated varieties used in the research have good morphological performance but the highest yield was recorded at maize OPV 3. Also, maize varieties planted at 0–15 cm depth of tillage had better performance and yield than those planted at 0–30 cm depth of tillage. Depths of tillage and varieties have effect on the performances and yield of yellow maize on the field.

Accepted for print 28.10.2021

References

ANDERSON J.A., D'SOUZA S. 2014. *From adoption claims to understanding farmers and contexts: A literature review of Conservation Agriculture (CA) adoption among smallholder farmers in southern Africa.* Agric. Ecosyst. Environ., 187(1): 116–132, https://doi.org/10.1016/j.agee.2013.08.008.

BERHANU S., MEBRATE M., ESSUBALEW G. 2016. *Effect of different sowing depth on germination and growth performance of maize (Zea mays* L.*) at Jimma, Southwest Ethiopia*. Int. J. Res. Innov. Earth Sci., 3(2): 50–55, http://www.ijries.org/administrator/components/com_jresearch/files/publications/IJAIR_ 1889_FINAL.pdf, access: 5.07.2021.

DONI S., MACCI C., LONGO V., SOUID A., GARCIA C., MASCIANDARO G. 2017. *Innovative system for biochemical monitoring of degraded soils restoration*. Catena, 152: 173–181. https://doi.org/10.1016/j.catena.2017.01.016.

EDMEADES G.O., BOLANOS J., ELINGS A., RIBAUT J.M., BANZIGER M., WESTGATE M.E. 2000. *The role and regulation of anthesis-silking interval in maize. Physiology and modelling kernel set in maize*. [In:] *Physiology and modeling kernel set in maize*. Ed. M.E. Westgate, K. Boote, D. Knievel, J. Kiniry, CSSA, Madison, pp. 43–73, https://doi.org/10.2135/cssaspecpub29.c4.

ENUJEKE E. 2013. *Effects of variety and spacing on yield indices of open-pollinated maize in Asaba Area of Delta State*. Journal of Sustainable Agriculture Research, 2(4): 1–11, https://doi.org/10.5539/sar.v2n4p1.

FAOSTAT. 2020. *Crop production statistics. Food and Agriculture Organization Data* http://www.fao.org/faostat/en/#data/QC.

HORTON M. 2019. *Reduced soil tilling helps both soils and yields*. Stanford News, https://news.stanford.edu/2019/12/06/reduced-soil-tilling-helps-soils-yields/, access: 8.12.2020.

KIM Y.S, KIM T.J, KIM Y.J, LEE S.D, PARK S.U., KIM W.S. 2020. *Development of a real-time tillage depth measurement system for agricultural tractors: application to the effect and analysis of tillage depth on draft force during plow tillage*. Sensors, 20(3): 912, https://doi.org/10.3390/s20030912.

MA S., YU Z., SHI Y., ZHANG Y., ZHAO J. 2015. *Effect of field border width for irrigation on dry matter accumulation and distribution, yield, and water use efficiency of wheat*. Acta Ecol. Sin., 35(6): 169–176, https://doi.org/10.1016/j.chnaes.2015.09.001.

MAGOROKOSHO C., PIXLEY K.V., TONGOONA P. 2003. *Selection for drought tolerance in two tropical maize populations*. African Crop Science Journal, 11(3): 151–161, http://www.bioline.org.br/pdf?cs03018.

MOJEED A. 2020. As *food shortage looms, Nigeria approves emergency importation of maize. Premium Times*, https://www.premiumtimesng.com/agriculture/agric-news/412263-as-food-shortage-looms-nigeria-approves-emergency-importation-of-maize.html#.YQE2YXqXYOE.link, access: 28.07.2021.

MOLATUDI R.L., MARIGA L.K. 2009. *The effect of maize seed size and depth of planting on seedling emergence and seed vigour*. J. Appl. Sci. Res., 5(12): 2234–2237, http://eprints.icrisat.ac.in/451/1/J.Appl.Sci.Res.5_12_2234_2237_2009.pdf.

MRABET R. 2011. *Effects of residue management and cropping systems on wheat yield stability in a semiarid Mediterranean clay soil*. Am. J. Plant Sci., 2(2): 202–216 http://dx.doi.org/10.4236/ajps.2011.22022.

NUEWEB. 2012. World Wheat Production, World Maize Production, World Rice http://nue.okstate.edu/Crop_Information/World_Wheat_Production.htm, access: 23.01.2021.

OLADEJO J.A., ADETUNJI M.O. 2012. *Economic analysis of maize (Zea mays* L.*) production in Oyo state of Nigeria*. Agric. Sci. Res. J. 2(2): 77–83, https://www.academia.edu/26060797/Economic_analysis_of_maize_zea_mays_l_product ion_in_Oyo_state_of_Nigeria.

PIAO L., QI H., LI C., ZHAO M. 2016. *Optimized tillage practices and row spacing to improve grain yield and matter transport efficiency in intensive spring maize*. Field Crops Res., 198: 258–268, https://doi.org/10.1016/j.fcr.2016.08.012.

PITTELKOW C.M., LIANG X, LINQUIST B.A., VAN GROENIGEN K.J., LEE J., LUNDY M.E., VAN GESTEL N., SIX J., VENTEREA R.T., VAN KESSEL C. 2015. *Productivity limits and potentials of the principles of conservation agriculture*. Nature, 517: 365–368. https://doi.org/10.1038/nature13809.

REN X., ZHANG P., CHEN X., GUO J., JIA Z. 2016. *Effect of different mulches under rainfall concentration system on corn production in the semi-arid areas of the loess plateau*. Sci. Rep., 6: 19019, https://doi.org/10.1038/srep19019.

Seid H., Brzegen A., Fentaye A. 2013. *Effect of planting depth on growth performance of maize (Zea-Mays) at the experimental site of Wollo University, Dessie, Ethiopia*. Int. J. Sci.: Basic Appl. Res., 8(1): 10–15, https://www.gssrr.org/index.php/JournalOfBasicAndApplied/article/view/1103/1100.

Shao Q., Baumgartl T. 2016. *Field evaluation of three modified infiltration models for the simulation of rainfall sequences*. Soil Sci. 181(2): 45–56, https://doi.org/10.1097/SS.0000000000000136.

Sun X., Ding Z., Hou H., Ge J., Tang L., Zhao M. 2013. *Post-anthesis photosynthetic assimilation and the changes of carbon and nitrogen in different varieties of spring maize*. Acta Agron. Sin., 39(7): 1284–1292, https://doi.org/10.3724/SP.J.1006.2013.01284.

Tamburini G., De Simone S., Sigura M., Boscutti F., Marini L., Kleijn D. 2016. *Conservation tillage mitigates the negative effect of landscape simplification on biological control*. J. Appl. Ecol., 53: 233–241, https://doi.org/10.1111/1365–2664.12544.

Wang S., Guo L., Zhou P., Wang X., Shen Y., Han H., Han K. 2019. *Effect of subsoiling depth on soil physical properties and summer maize (Zea mays L.) yield*. Plant Soil Environ., 65(3): 131–137, https://doi.org/10.17221/703/2018-PSE.

Wato T. 2019. *The effect of planting depth on germination and seedling vigoursity of maize (Zea mays L.)*. Int. J. Res. Anal. Rev., 6(2): 840–845, http://dx.doi.org/10.13140/RG.2.2.14279.24485.

Wits 2021. *Nigeria Cereal flour; of maize (corn) imports by country in 2018*, https://wits.worldbank.org/trade/comtrade/en/country/NGA/year/2018/tradeflow/Imports/partner/ALL/product/110220, access: 28.07.2021.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 229–250, Y. 2021

ASSESSMENT OF MACROINVERTEBRATE COMMUNITY IN A RURAL NIGERIAN RIVER IN RELATION TO ANTHROPOGENIC ACTIVITIES

***Emeka Donald Anyanwu*[1], *Sabastine Nnanna Odo*[2], *Uchechi Augusta Nwaiwu*[3]**

[1] ORCID: 0000-0002-8593-6865
[2] ORCID: 0000-0002-5386-7818
[1,3] Department of Zoology and Environmental Biology
[2] Department of Fisheries and Aquatic Resources Management
Michael Okpara University of Agriculture, Umudike, Nigeria

Key words: water quality, sand mining, macroinvertebrate, biodiversity, CCA.

Abstract

An assessment of macroinvertebrate community and water quality of a rural river in South-East Nigeria was carried out between May and October 2019 in three stations in relation to anthropogenic activities. The major anthropogenic activity in the river was indiscriminate sand mining. The water samples were collected and analyzed using standard methods while macroinvertebrates were sampled using modified kick method and sweeping of aquatic macrophytes with handnet. Results showed that pH, dissolved oxygen and biochemical oxygen demand were not within acceptable limits. 346 macroinvertebrates individuals from 12 taxa and 3 taxonomic groups were recorded. The macroinvertebrates consist more of pollution tolerant species like *Haliplus* sp. larvae and *Chironomus* sp. larvae and the community structure pointed to perturbation. Canonical correspondence analysis showed that the first canonical axis accounted for over 80% of the variation with eigen value of 0.042. Axis 1 had strong positive association with Hemiptera and Diptera explained by temperature and BOD while Axis 2 had strong positive association with Araneae influenced by phosphate and BOD. Station 2 influenced by temperature, had strong positive association with Axis 1 while station 1 influenced by pH, TDS and EC, had strong negative association with Axis 1. Station 3 influenced by phosphate and BOD had strong positive association with Axis 2. Indiscriminate sand mining have not adversely affected the water quality; however, the macroinvertebrate community was adversely affected which reflected in the dominance of tolerant species and the community structure.

Address: Emeka Donald Anyanwu, Michael Okpara University of Agriculture, Umudike, Nigeria, phone: +2347036373209, e-mail: ekadon@yahoo.com

Introduction

Rivers as important biodiversity systems are among the most productive ecosystems on the earth and provide the favourable conditions that support wide range of flora and fauna. Most of the freshwater bodies all over the world are polluted as a result of human activities and thus affecting the ecosystem services derivable from them (GUPTA et al. 2005, ANYANWU 2012, GOLDSCHMIDT 2016, AMAH-JERRY et al. 2017). The quality of water can be affected by meteorological and climatic factors while the variability of anthropogenic activities was the major factor that could explain the day to day variability (SCHEILI et al. 2016a, b). Indiscriminate sand mining is a major anthropogenic activity in most rivers in the region (including Iyiakwu River), which was observed to increase with increasing rainfall (ANYANWU and UMEHAM 2020). Indiscriminate sand mining has been reported as one of the potential threats to the freshwater biota; through increased turbidity, reduced organic detritus supply, loss of breeding and spawning grounds (SHEEBA 2009). The quality of the aquatic ecosystem can be predicted by the assessment of biological communities and many researchers have used this approach to determine the ecological effects of pollution (AKINDELE and LIADI 2014, ANYANWU et al. 2019, ALIU et al. 2020, SANTOS and FERREIRA 2020). The Nigerian inland waters have been reported to support a wide range of aquatic organisms (ATOBATELE and UGWUMBA 2008).

The freshwater macroinvertebrates are largely made up of insects; others include crustaceans, gastropods, bivalves and oligochaetes (ALLAN 1995, MERRITT et al. 2008, THORP and COVICH 2001). The use of macroinvertebrates in biological monitoring have been consistent and reliable when compared to the use of other organisms (plankton, fish, etc) because they have wide distribution, sensitive to organic pollutants and easy to sample at minimal cost (KALYONCU and GULBOY 2009, SETIAWAN 2009). Factors like water quality, substrate type, sediment and particle size, flow regime determine the community structure of macroinvertebrates in association with the prevailing conditions in the watershed (WARD et al. 1995, BUSS et al. 2004).

A number of studies on macroinvertebrate communities and water quality have been carried out in Nigeria (ARIMORO et al. 2015, ANYANWU and JERRY 2017, IYAGBAYE et al. 2017, ANYANWU et al. 2019, ALIU et al. 2020). However, little attention has been given to the best of my knowledge to smaller rural rivers like Iyiakwu, scattered all over the country and harbour a significant proportion of the nation's freshwater biodiversity.

The objective of this study was to assess the macroinvertebrate community and water quality of Iyiakwu River, Elemaga, Ikwuano, South-East Nigeria in relation to anthropogenic activities.

Materials and Methods

Study area and sampling stations

The study was carried out in Iyiakwu River, Elemaga, Ikwuano Local Government Area, Abia State, Nigeria. The section of the river studied lies within Latitude 05°26'21"–05°26'40"N and Longitude 07°37'3"–07°37'16"E (Figure 1). The study area is within the sub-equatorial zone; having a mean annual rainfall of 4000 mm (NWANKWO and NWANKWOALA 2018). It is characterized by high relative humidity of over 70% and high temperature of about 29–31°C (NWANKWO and NWANKWOALA 2018). It is also characterized by the wet season (May to October) and dry season (November to April); a double maxima rainfall peaks in July and October. The area also experiences a short period of dryness between the peaks in August usually referred to as "August break".

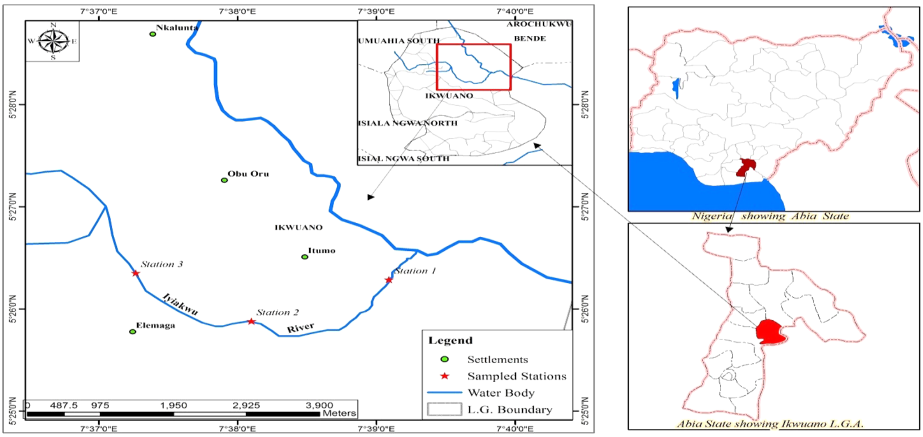


Fig. 1. Map of Elemaga, Ikwuano LGA, Abia State, Nigeria showing the sampling stations of Iyiakwu River

UHEGBU et al. (2013) reported that the area is situated geologically in the Eastern Niger Delta; falling within Bende-Ameki Formation and the Benin Formation in Abia State. The Bende-Ameki formation was further classified into two lithological groups – the lower part made up of fine to coarse grain sandstones and intercalations of calcareous shales and thin shelly limestone and upper part made up of coarse, cross-bedded sandstone with bands of fine, grey-green sandstone and sandy fossiliferous clays (FMENV. 2005). The age of the formation has been reported to be early Eocene and early middle Eocene respectively (UHEGBU et al. 2013, OBASI et al. 2015). The area is endowed with natural springs and streams including Iyiakwu River which flows from Bende (the northern boundary) through the study area in a south-westerly direction and empties itself at the Qua Iboe river in Akwa Ibom state (UKAGWU et al. 2017, NWANKWO and NWANKWOALA 2018)

Station 1 was upstream, located in an agricultural area with sandy substrate. Sand mining activities were observed some distances upstream of this station. Human activities observed during the study include extraction of water for drinking, processing of breadfruit (*Treculia africana*) and fermentation of cassava (*Manihot esculenta*) tubers in plastic containers and farming activities. Station 2 was 2.15 km downstream of station 1 with sandy substrate. Sand mining activities was intense; other activities include extraction of water for drinking and nursery, washing of clothes, fermentation and processing of cassava (*Manihot esculenta*) tubers in plastic containers and swimming. Station 3 was 1.97 km downstream of station 2; also with sandy substrate. It was located within a large expanse of palm bush, cocoa farms and farmlands. Little or no activities were observed during the study but sand mining activities was observed in September and October 2019.

Samples Collection and Analyses

Water samples

Water samples were collected monthly from Iyiakwu River between May and October 2019 in sterilized 1litre plastic bottles. Some physicochemical parameters were determined *in-situ* – water temperature was determined with mercury-in-glass thermometer, flow velocity was determined by floatation method, transparency was determined with Secchi Disk, pH, electrical conductivity and total dissolved solids were all determined with handheld meter (pH/EC/TDS Meter-HANNA 3100 Model). Other parameters were determined in the laboratory – dissolved oxygen and biochemical oxygen demand were determined with Winkler's Method with azide modification method while nitrate was determined with UV spectrophotometric method and phosphate with stannous chloride method.

Macroinvertebrate sampling

Macroinvertebrate samples were collected from the sediments using the modified kick sampling technique described by KEÇI et al. (2012). The sediment upstream was disturbed by kicking with foot for about 5 minutes and the macroinvertebrates dislodged were washed into the net placed downstream of the disturbed point. Aquatic macrophytes along the banks of the river were also swept with the hand net against the water current and the macroinvertebrates dislodged were washed into the net. All the samples were preserved with 10% formalin in a plastic container and taken to the laboratory for proper identification. The isolated macroinvertebrates were identified to the lowest possible taxonomic level with the aids of the following taxonomic keys: WILLOUGHBY (1976), MERRITT and CUMMINS (1996) and UMAR et al. (2013). The numbers were counted.

Statistical Analysis

The data were summarised into maximum, minimum, mean and standard error of the mean using Descriptive Statistic Package of Microsoft Excel while two-way ANOVA was used to test for statistical differences among the stations and months. Tukey's pairwise comparisons test was performed to determine the location of significant difference ($P < 0.05$). The community structure of macroinvertebrates was determined using Margalef (D), Shannon-Wiener (H) and Evenness (E) indices. Canonical correspondence analysis (CCA) was used to evaluate relationships between the macroinvertebrate groups and environmental variables with PAST statistical package (HAMMER et al. 2001).

Results

Water quality

The summary of physico-chemical parameters measured in the surface water of Iyiakwu River is shown in Table 1. Two-way analysis variance (ANOVA) showed that there were no significant differences ($P > 0.05$) in all the physicochemical parameters evaluated.

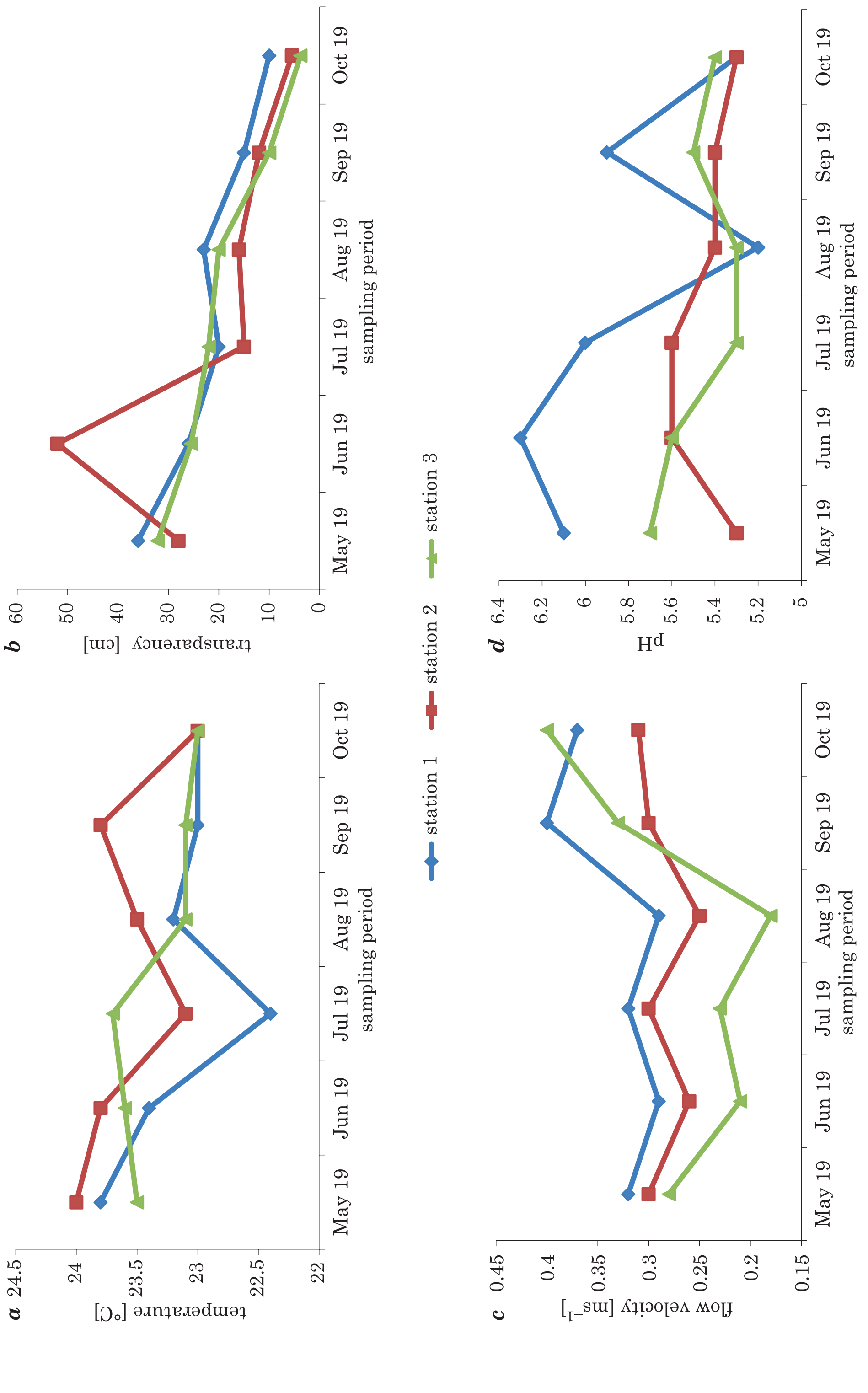
Water temperature values ranged between 22.4 and 24.0°C. The lowest water temperature value was recorded in station 1 in July 2019 while the highest water temperature value was recorded in station 2 in May 2019 (Figure 2*a*). The transparency values ranged between 3.8 and 52.0 cm. The lowest transparency value was recorded in station 3 in October 2019 while the highest transparency value was recorded in station 1 in June 2019 (Figure 2*b*). Transparency generally decreased with increasing rainfall from May to October 2019. The flow velocity values ranged between 0.18 and 0.40 ms^{-1}. The lowest flow velocity value was recorded in station 2 in August 2019 while the highest flow velocity value was recorded in station in 3 in September 2019 (Figure 2*c*). The flow velocity generally increased with the rains from August 2019.

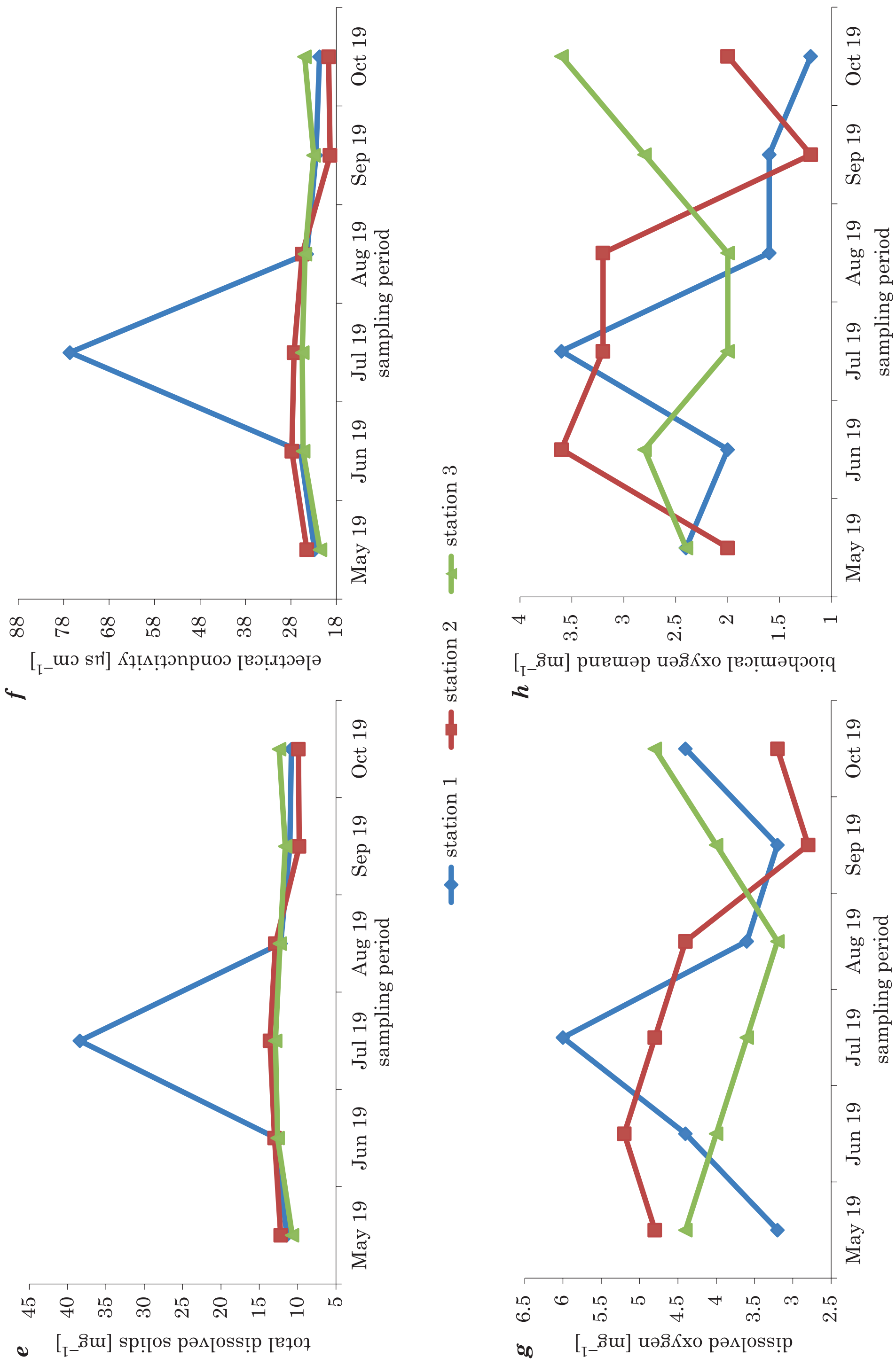
Table 1

Summary of some physico-chemical parameters of Iyiakwu River (with range in parenthesis)

Parameter	Station 1 X±SEM	Station 2 X±SEM	Station 3 X±SEM	Station P-value	Month P-value	FMEnv.
Water temperature [^oC]	23.1±0.19 (22.4–23.8)	23.5±0.17 (23.0–24.0)	23.3±0.12 (23.0–23.7)	0.336	0.353	–
Transparency [cm]	21.7±3.69 (10.0–36.0)	21.4±6.80 (5.5–52.0)	18.9±4.22 (3.8–32.1)	0.637	0.073	–
Flow velocity [ms^{-1}]	0.33±0.02 (0.29–0.40)	(0.28±0.01) 0.25–0.31	(0.27±0.03) 0.18–0.40	0.590	0.155	–
pH	5.8±0.18 (5.2–6.3)	5.4±0.06 (5.3–5.6)	5.5±0.07 (5.3–5.7)	0.741	0.671	6.5–8.5
Total dissolved solids [mg l^{-1}]	16.1±4.47 (10.8–38.4)	11.9±0.67 (9.8–13.6)	12.1±0.32 (10.7–12.9)	0.788	0.291	–
Conductivity [µS cm^{-1}]	32.3±8.87 (21.7–76.6)	24.0±1.50 (19.4–27.8)	24.2±0.64 (21.6–25.5)	0.901	0.288	–
Dissolved oxygen [mg l^{-1}]	4.1±0.43 (3.2–6.0)	4.2±0.40 (2.8–5.2)	4.0±0.23 (3.2–4.8)	0.718	0.746	> 6
Biochemical oxygen demand [mg l^{-1}]	2.1±0.35 (1.2–3.6)	2.5±0.38 (1.2–3.6)	2.6±0.25 (2.0–3.6)	0.907	0.821	3
Nitrate [mg l^{-1}]	0.19±0.02 (0.15–0.31)	0.19±0.01 (0.14–0.24)	0.17±0.01 (0.12–0.21)	0.269	0.105	50
Phosphate [mg l^{-1}]	0.11±0.02 (0.09–0.20)	0.11±0.01 (0.08–0.15)	0.11±0.01 (0.09–0.13)	0.629	0.122	3.5

SEM – Standard Error of Mean; FMEnv. – Federal Ministry of Environment (2011)


a
temperature [°C]
24.5
24
23.5
23
22.5
22
b
transparency [cm]
60
50
40
30
20
10
0
c
flow velocity [ms^{-1}]
0.45
0.4
0.35
0.3
0.25
0.2
0.15
d
pH
6.4
6.2
6
5.8
5.6
5.4
5.2
5
May 19
Jun 19
Jul 19
Aug 19
Sep 19
Oct 19
sampling period
station 1
station 2
station 3


e
total dissolved solids [mg^{-1}]
f
electrical conductivity [μs cm^{-1}]
g
dissolved oxygen [mg^{-1}]
h
biochemical oxygen demand [mg^{-1}]
May 19
Jun 19
Jul 19
Aug 19
Sep 19
Oct 19
sampling period
station 1
station 2
station 3

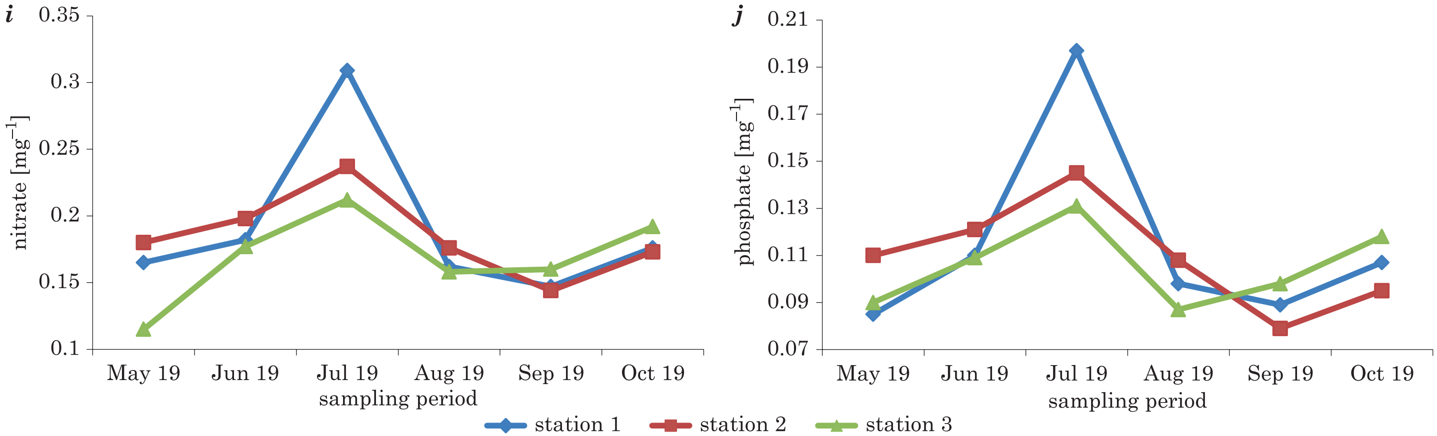


Fig. 2. Spatio-temporal variations of: *a* – temperature; *b* – transparency; *c* – flow velocity; *d* – pH; *e* – total dissolved solids; *f* – electrical conductivity; *g* – dissolved oxygen; *h* – biochemical oxygen demand; *i* – nitrate; *j* – phosphate in Iyiakwu River, South-East Nigeria in 2019

pH values ranged between 5.2 and 6.3. The lowest pH value was recorded in station 1 in August 2019 while the highest pH was recorded in station 1 in June 2019 (Figure 2*d*). All pH values were acidic and outside the acceptable limit set by FMEnv. (2011). Electrical conductivity values ranged between 19.4 and 76.6 μS cm^{-1}. The lowest conductivity value was recorded in station 3 in September 2019 while the highest conductivity value was recorded in station 1 in July 2019 (Figure 2*e*). Total dissolved solid values recorded ranged between 9.8 and 38.4 mg l^{-1}. The lowest TDS value was recorded in station 2 in September while the highest TDS value was recorded in station 1 in July 2019 (Figure 2*f*). TDS followed the same trend as Electrical Conductivity. Dissolved oxygen values ranged between 2.8 and 6.0 mg l^{-1}. The lowest DO value was recorded in station 3 in September 2019 while the highest DO value was recorded in station 2 in July 2019 (Figure 2*g*).

All DO values were lower than acceptable limit, except one in station 1 (July 2019). Biochemical Oxygen Demand values ranged between 1.2 and 3.6 mg l^{-1}. The lowest BOD value was recorded in station 1 in September 2019 while the highest BOD values were recorded in stations 1, 2 and 3 in July, June and October 2019 respectively (Figure 2*h*). These values exceeded limit set by FMEnv. (2011).

Nitrate values ranged between 0.12 and 0.31 mg l^{-1}. The lowest nitrate value was recorded in station 3 in May 2019 while the highest nitrate value was recorded in station 1 in July 2019 (Fig. 2*i*). Station 1 was generally higher than the others though within acceptable limit. The phosphate values ranged between 0.08 and 0.20 mg l^{-1}. The lowest phosphate value was recorded in station 2 in September 2019 while the highest phosphate value was recorded in station 1 in July 2019 (Figure 2*j*). All the phosphate values were within acceptable though station 1 recorded higher values.

Macroinvertebrates Composition, Abundance and Distribution

The overall species composition, abundance and distribution of macroinvertebrates are presented in Table 2. A total of 346 macroinvertebrate individuals comprising of three (3) taxonomic groups and twelve (12) taxa was recorded. Percentage composition of the taxa showed that freshwater shrimp, *Caridina africana* had the highest abundance (24%), followed by crawling water beetle, *Haliplus* sp. larvae (16.8%) while the least was the water strider (*Aquarius remigis*). All the macroinvertebrates recorded in this study were tolerant species except *Caridina africana*.

Spatially, station 3 recorded the highest number of macroinvertebrate individuals (127), followed by station 1 (116 individuals) and the least is station 2 (103 individuals). Monthly, the highest number of individuals

(31) was recorded in May and June 2019 while the lowest (3 individuals) was recorded in October 2019 (station 1). In station 2, the highest number of individuals (35) was recorded in May 2019 while the lowest (4 individuals) was recorded in October 2019. The highest number of individuals recorded in station 3 was 29 in July 2019 while the lowest (5 individuals) was also in October 2019 (Figure 3).

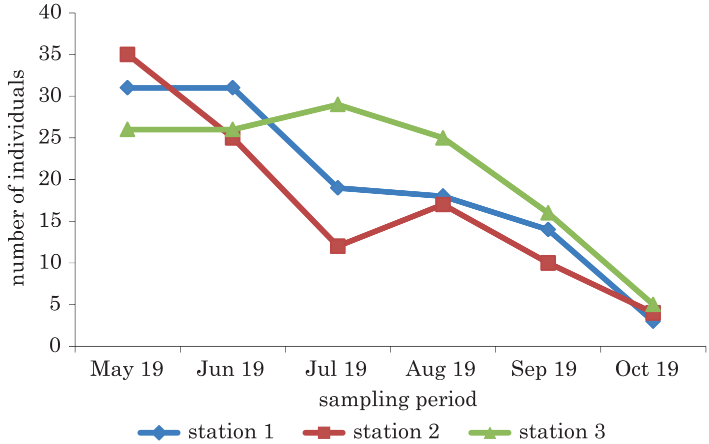


Fig. 3. Monthly distribution of macroinvertebrate individuals in Iyiakwu River, South-East Nigeria

Table 2

Species composition, abundance and distribution of macroinvertebrate fauna encountered in Iyiakwu River, Elemaga, Southeast Nigeria

Group	Taxa	Stn 1	Stn 2	Stn 3	Total	Percent-age
Insecta Hemiptera	*Aquarius remigis* (Say, 1832)	1	4	4	9	2.6
Diptera	*Chironomus* sp. (Meigen, 1803)	3	15	11	29	8.4
	Anopheles gambiae (Giles, 1902)	3	4	5	12	3.5
Coleoptera	*Haliplus* sp. *Larvae* (Stephens, 1821)	17	19	22	58	16.8
	Agabus bipustulatus (Linnaeus, 1767)	4	5	7	16	4.6
	Hydrophilis triangularis (Say, 1823)	10	5	9	24	6.9
Odonata	*Libellula* sp. nymph (Linnaeus, 1758)	10	6	8	24	6.9
	Gomphus sp. nymph (Charpentier, 1825)	9	7	8	24	6.9
	Macromia sp. nymph (Walsh, 1862)	8	9	6	23	6.7
Crustacea Decapoda	*Sudanonautes africanus* (A. Milne-Edwards, 1869)	9	8	10	27	7.8
	Caridina africana (H. Milne-Edwards, 1837)	37	18	28	83	24
Arachnida Araneae	*Argyroneta aquatica* (Clerck, 1758)	5	3	9	17	4.9

The community structure showed that Margalef species richness index had a narrow range of between 2.271 and 2.273 (Table 3). On the other hand, Shannon-Wiener index ranged from 2.131 to 2.313; with the highest value recorded in station 3. The highest Evenness index (0.8418) was also recorded in station 3.

Table 3

Biodiversity indices of macroinvertebrate fauna of Iyiakwu River, South-East Nigeria

Biodiversity indices	Stn 1	Stn 2	Stn 3
Taxa (S)	12	12	12
Individuals	116	103	127
Shannon-Wiener (*H*)	2.131	2.306	2.313
Evenness (E)	0.7021	0.8362	0.8418
Margalef (D)	2.314	2.373	2.271

Relationship between macroinvertebrate and physico-chemical parameters

The canonical correspondence analysis (CCA) ordination also showed a good relationship between the macroinvertebrate groups and environmental variables. The first canonical axis accounted for over 80% of the variation in the data set (Table 4). Axis 1 had strong positive association

Table 4

Correlations of environmental variables with the two axes of canonical correspondence analysis (CCA)

Variables	Axis 1	Axis 2
Eigenvalue	0.041576	0.009274
Variation of species data explained [%]	81.76	18.24
HEM	0.483973	0.065333
DIP	0.445396	-0.0415
ARA	-0.0433	0.352332
Station 1	-1.31265	-0.50962
Station 2	1.15547	-1.01371
Station 3	0.261842	1.28763
Temperature	0.987553	-0.20835
pH	-0.99243	-0.07109
TDS	-0.94815	-0.26817
EC	-0.94123	-0.28843
BOD	0.849489	0.482787
PO4	-0.35756	0.951195

with Hemiptera and Diptera; explained mostly by temperature and BOD while Axis 2 also had strong positive association with Araneae that was mostly explained by phosphate and BOD (Table 4, Figure 4). Spatially, Axis 1 had strong positive association with station 2; explained mostly by temperature and strong negative association with station 1; explained by pH, TDS and EC. On the other hand, Axis 2 had strong positive association with station 3 that was mostly explained by phosphate and BOD (Table 4, Figure 4).

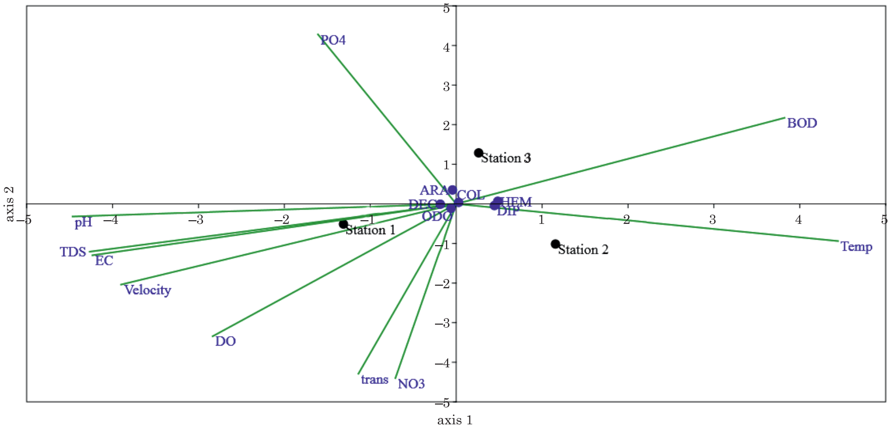


Fig. 4. Canonical correspondence analysis (CCA) ordination showing relationships between macroinvertebrate groups, sites and environmental variables (BOD – biochemical oxygen demand, DO – dissolved oxygen; trans – transparency; temp – water temperature; NO_3 – nitrates; PO_4 – phosphates; EC – electrical conductivity; Ara – araneae; Col – coleoptera; Hem – hemiptera; Dec – decapoda; Odo – Odonata; Dip – diptera)

Discussion

Water quality

All the water temperature values were low though station 2 recorded slightly higher temperatures which could be attributed to the openness of the station; the other stations were shaded by trees. According to Mohseni and Stefan (1999) and Webb et al. (2003), air temperatures influence water temperatures; the lowest water temperature was recorded in station 1 in July, which is one of the peaks of wet season while the highest value was recorded in station 2 in May before the onset of the wet season. The temperatures values recorded in this study were relatively lower compared to values recorded elsewhere in Nigeria. Abdullahi et al. (2008) recorded 27.7–29.9°C in Challawa River, Kano, Akpe et al. (2018) recorded 23.0–30.5°C in Ikpoba River, Benin City and Anyanwu and Mbekee (2020) recorded 21.0–28.0°C in Ossah River, Umuahia, Nigeria.

The lowest transparency was recorded in station 3 in October; attributable to season and mining activities which peaked in September and October 2019 while the highest was recorded in station 1 before the early rains in June 2019. Anthropogenic activities like sand mining reduce transparency and light penetration (KALE 2016) and have adverse effect on benthic community and other aquatic organisms (ESENOWO and UGWUMBA 2010). The transparency values were low compared to 12.5–100 cm recorded by ANYANWU (2012) in Ogba River, Benin City and 50.0–94.0 cm recorded by ANYANWU and MBEKEE (2020) in Ossah River, Umuahia, Nigeria.

The flow velocities were moderate and generally increased with the rains. The lowest value was recorded in station 2 during the August break while the highest values were recorded in stations 1 and 3 during the peaks of rain (September and October 2019 respectively). The flow velocities were lower than 1.48–1.83 ms^{-1} recorded in selected river in Ebonyi State, South-East Nigeria by ANI et al. (2016) but higher than 0.05–0.13 ms^{-1} recorded by ANYANWU and MBEKEE (2020) in Ossah River, Umuahia, Nigeria. Different taxa are known to cope with different flow velocity thresholds and time spans of being exposed to increased discharge (BROOKS et al. 2005, OLDMEADOW et al. 2010). The velocity of water body also influences the water body ability to assimilate and remove water pollutants (EFFENDI et al. 2015).

The pH values were acidic and not within acceptable limit; attributed to the geogenic, seasonal and anthropogenic influences. The values were within the range recorded in the related studies in the region. ANYANWU et al. (2019) recorded a range of 4.6–6.3 in Ossah River, Umuahia and ANYANWU and UMEHAM (2020) recorded a range of 4.9–6.3 in Eme River, Umuahia, Nigeria. However, AMAH-JERRY et al. (2017) recorded a higher range of 5.0–9.1 in Aba River, Aba. The lowest value was recorded in station 1 during the August break attributed to low precipitation and higher atmospheric temperatures leading to concentration while the highest value recorded in station 1 during the onset of rains in June may be due to dilution by rain water (ATOBATELE and OLUTONA 2013, ETESIN et al. 2013, HOUSSOU et al. 2017). Sand mining activities also reduce the pH level of water bodies as observed in ANYANWU et al. (2019) and ANYANWU and UMEHAM (2020). Aquatic organisms are affected by pH because most of their metabolic activities are pH dependent (MANICKAM et al. 2015).

The electrical conductivity values were moderate. Related studies in the region recorded close ranges. AMAH-JERRY et al. (2017) recorded a range of 3.5–98.0 $\mu S\ cm^{-1}$ in Aba River, Aba and ANYANWU et al. (2019) recorded a range of 52.9–110.5 $\mu S\ cm^{-1}$ in Ossah River, Umuahia, Nigeria.

The lowest value was recorded in station 3 in September 2019 which could be attributed to dilution by increased rainfall while the highest was recorded in station 1 in July which could be attributed to intense sand mining activities around the station. The highest value recorded in ANYANWU et al. (2019) was in station 1 that was subjected to sand mining activities. REHMAN et al. (2016) and AKANKALI et al. (2017) reported that sand mining activities can contribute to the increase in EC values while DORAK (2013) pointed out that increasing EC is an indication of increasing pollution

Total Dissolved Solids values were within acceptable limits and followed the same trend with Electrical conductivity. AMAH-JERRY et al. (2017) recorded a range of 8.0–24.2 mg l^{-1} in Aba River, Aba and ANYANWU et al. (2019) recorded a range of 25.7–55.3 mg l^{-1} in Ossah River, Umuahia, Nigeria. EWA et al. (2011) reported that high level of EC usually correspond to high value of TDS. Sand mining activities can contribute to the increase in TDS as observed in electrical conductivity.

Most of the Dissolved Oxygen values were lower than acceptable limit. This trend was observed in related studies in the region. AMAH-JERRY et al. (2017) recorded a higher range of 2.7–8.8 mg l^{-1} in Aba River, Aba, ANYANWU et al. (2019) recorded a range of 3.2–6.4 mg l^{-1} in Ossah River, Umuahia and ANYANWU and UMEHAM (2020) recorded a range of 1.6–6.1 mg l^{-1} in Eme River, Umuahia, Nigeria. The lowest value was recorded in station 3 in September 2019 while the highest value was recorded in station 2 in July 2019. Some of the impacts associated with sand mining activities like addition of nutrients and chemicals, altering the flow of water and raising the water temperature can reduce the oxygen content of water (RAO et al. 2013). The lowest DO value recorded in ANYANWU and UMEHAM (2020) was in station 4, which was immediately downstream to active sand mining and sand landing sites.

Some BOD values were above the acceptable limit. This trend was observed in related studies in the region. AMAH-JERRY et al. (2017) recorded a higher range of 1.1–6.1 mg l^{-1} in Aba River, Aba, ANYANWU et al. (2019) recorded a range of 1.5–4.2 mg l^{-1} in Ossah River, Umuahia and ANYANWU and UMEHAM (2020) recorded a range of 0.8–4.3 mg l^{-1} in Eme River, Umuahia, Nigeria. Relatively higher values were recorded in stations 1, 2 and 3 in July, June and October 2019 respectively, which could be associated with sand mining activities. This agreed with AKANKALI et al. (2017), ANYANWU et al. (2019) and ANYANWU and UMEHAM (2020).

Nitrate values were all within acceptable limit. The values were lower than the range recorded in the related studies in the region. AMAH-JERRY et al. (2017) recorded a higher range of 7.4–79.8 mg l^{-1} in Aba River, Aba, ANY-

ANWU et al. (2019) recorded a range of 0.9–3.4 mg l^{-1} in Ossah River, Umuahia and ANYANWU and UMEHAM (2020) recorded a range of 1.1–5.6 mg l^{-1} in Eme River, Umuahia, Nigeria. The lowest value was recorded in station 3 before the onset of rains in May 2019 while the highest was recorded in station 1 during one of the peaks in July 2019. Station 1 was generally higher than the others though within acceptable limit, which could be attributed to the farming activities in the watershed rather than sand mining activities. SOLANKI (2012) reported that runoffs from agricultural fields is one of the major sources of nitrate inputs into freshwater. This is contrary to ANYANWU et al. (2019) and ANYANWU and UMEHAM (2020) where the highest values were recorded in stations subjected to sand mining activities.

All the phosphate values were within acceptable limit though station 1 recorded higher values. The values were lower than the range recorded in the related studies in the region. AMAH-JERRY et al. (2017) recorded a higher range of 2.3–79.8 mg l^{-1} in Aba River, Aba, ANYANWU et al. (2019) recorded a range of 0.1–2.7 mg l^{-1} in Ossah River, Umuahia and ANYANWU and UMEHAM (2020) recorded a range of 0.4–4.6 mg l^{-1} in Eme River, Umuahia, Nigeria. The lowest value was recorded in station 2 in September 2019; attributable to dilution while the highest was recorded in station 1 in July 2019; attributable to agricultural activities as in the case of nitrate. Use of fertilizers and pesticides are some of the sources of phosphate in water system (MANDEL et al. 2012). This is also contrary to ANYANWU et al. (2019) and ANYANWU and UMEHAM (2020) where the highest values were recorded in stations subjected to sand mining activities.

Macroinvertebrates

The macroinvertebrate species composition was dominated by group insecta as in ANYANWU et al. (2019) but different from the dominance of group mollusca in ANYANWU and JERRY (2017). The number of individuals was higher, taxonomic groups lower and taxa was higher when compared to 168 individuals, 4 taxonomic group and 7 taxa recorded by ANYANWU and JERRY (2017) in a suburban River in Umuahia, Southeast Nigeria. ANYANWU et al. (2019) also recorded lower number of individuals (119), higher taxonomic groups (5) and taxa (20) in an effluent-receiving river in Umuahia, Southeast Nigeria.

The number of individuals, taxonomic groups and taxa were affected by sand mining activities as reflected in the community structure. ZOU et al. (2019) reported reductions of 89.80% and 99.54%, respectively in the macroinvertebrate density and biomass, a significant reduction in the majority of macroinvertebrate taxonomic groups and species due substrate destruction and high turbidity associated with sand mining. SHEEBA (2009) also reported indiscriminate sand mining as one of the potential threats to the freshwater biota; through increased turbidity, reduced organic detritus supply, loss of breeding and spawning grounds.

The macroinvertebrate species recorded had some pollution tolerant species especially *Haliplus* sp larvae and *Chironomus* sp larvae. Tolerant organisms can survive in unstable environment because of their ability to cope with perturbations (MARIANTIKA and RETNANINGDYAH 2014) and become more abundant (KUCUK 2008). ZOU et al. (2019) recorded a decrease of 28% in macroinvertebrate density and an increase in the density of crustacea due to high turbidity in an area adjacent to the sand mining site. Decapod crustacean, *Caridina africana* was recorded in high numbers in all the stations. Station 3 had the highest number of individuals while station 2 had the lowest which could be as a result of the effect of sand mining activities in the station (SHEEBA 2009, ZOU et al. 2019). Benthic forms are severely destroyed and their re-colonization prevented by sand mining activities (BHATTACHARYA 2018). Macroinvertebrate abundance decreased temporally with increase in the amount of rainfall. MCCABE (2010) reported reduction of macroinvertebrate abundances by half or more as being usual after heavy rainfalls. The decrease could also be attributed to sand mining activities because the intensity usually increases with the rains (ANYANWU and UMEHAM 2020). It has been reported that rivers with unstable substrates tend to have low species diversity and the organisms present usually manifest characteristics of disturbed environments (COBB et al. 1992). Invertebrate drifting could also be responsible (NAMAN et al. 2016). Invertebrate drift could result from a number of factors; CASTRO et al. (2013) listed accidental dislodgement from the substratum, interaction with other organisms, water quality changes, discharge and current velocity. Some of these could be triggered by sand mining activities. KOEHNKEN et al. (2020) reported that sand mining affect macroinvertebrate drift, species abundance and community structures among others. The macroinvertebrate community structure showed signs of perturbation. BALLOCH et al. (1976) reported that the diversity indices can be used as suitable indicators of water quality. Species diversity indices are useful tools for comparing communities to identify biotic disturbances or level of stability (OLAWUSI-PETERS and AJIBARE 2014) and it increases as the habitat increases in complexity or stability (LEINSTER and COBBOLD 2012). The Shannon-Wiener diversity values recorded were in the range indicating perturbation especially in station 2. The Shannon-Wiener diversity index is usually from 1.5 to 3.5 and hardly exceeds 4.5 (MAGURRAN

1988, Bibi and Ali 2013). The index categories showed that values of < 1 indicated heavily polluted conditions, values of 1 to 2 indicated moderate polluted conditions and values of > 3 indicated stable environmental conditions (Stub et al. 1970, Mason 2002). The Margalef indices were equally low, also indicating environmental instability. Margalef index does not have upper limit value and varies according to the number of species. The pielou's measure of evenness was reduced especially in station 1. Leinster and Cobbold (2012) reported that evenness is an important aspect of diversity indices indicating the even distribution of the individuals within the different species. Shannon-Wiener diversity and evenness indices were slightly higher in station 3 indicating signs of possible recovery.

Conclusion

Certain anthropogenic activities have more adverse impacts on water quality and the biota than the others. This study has shown that indiscriminate sand mining have not adversely affected the water quality. However, the macroinvertebrate community was adversely affected which reflected in the presence of tolerant species and the community structure. The community structure gave an insight into the negative impact of sand mining. There is need to regulate sand mining in order to preserve the biotic components of such rural waterbodies.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

Authors would like to thank Mr. Tom Akan of Department of Geography, University of Uyo, Uyo, Nigeria for producing the study map and Mr. Emmanuel Ogbonna Irozuru of Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Nigeria for assisting in the identification of the macroinvertebrate samples.

Accepted for print 1.09.2021

Reference

ABDULLAHI B.A., KAWO A.H., NA'ALIYA J. 2008. *Observations on the seasonal and spatial variations in water quality and ecological implications of Challawa River, Nigeria*. Biosci. Res. Commun., 20(3): 121–126.

AKANKALI J.A., IDONGESIT A.S., AKPAN P.E. 2017. *Effects of sand mining activities on water quality of Okoro Nsit stream, Nsit Atai Local Government Area, Akwa Ibom State, Nigeria*. Int. J. Dev. Sustain., 6: 451–462.

AKINDELE E.O., LIADI A.A. 2014. *Diversity and response of benthic macroinvertebrates to natural and induced environmental stresses in Aiba Stream, Iwo, Southwestern Nigeria*. West Afr. J. Appl. Ecol., 22: 101–111.

AKPE A. R., FEMI I. J., OKWU G. I., OBIAZI H. 2018. *Seasonal variations in the physicochemical parameters of Ikpoba River Water Samples*. Int. J. Life. Sci. Scienti. Res., 4(3): 1810–1821

ALIU O.O., AKINDELE E.O., ADENIYI, I.F. 2020. *Biological assessment of the Headwater Rivers of Opa Reservoir, Ile-Ife, Nigeria, using ecological methods*. J. Basic Appl. Zool., 81: 11, https://doi.org/10.1186/s41936-020-00151-5.

ALLAN J.D. 1995. *Stream ecology. Structure and function of running waters*. Springer Netherlands.

AMAH-JERRY E.B., ANYANWU E.D., AVOAJA D.A. 2017. *Anthropogenic impacts on the water quality of Aba River, Southeast Nigeria*. Eth. J. Environ. Stud. Manage., 10(3): 299–314, doi: https://dx.doi.org/10.4314/ejesm.v10i3.3.

ANI C., OKOGWU O.I., NWONUMARA G.N., NWANI C.D., NWINIYIMAGU A.J. 2016. *Evaluation of physicochemical parameters of selected rivers in Ebonyi State, Southeast, Nigeria*. Greener J. Biol. Sci., 6(2):34–41.

ANYANWU E.D. 2012. *Physicochemical and some trace of metal analysis of Ogba River, Benin City, Nigeria*. Jordan J. Biol. Sci., 5(1): 47–54.

ANYANWU E.D., JERRY P.O. 2017. *A Survey of macroinvertebrate assemblage of Ikwu River, Umuahia, Southeast Nigeria*. J. Aquat. Sci., 32(1A): 45–51. doi: https://dx.doi.org/10.4314/jas.v32i1A.5.

ANYANWU E.D., OKORIE M.C., ODO S.N. 2019. *Macroinvertebrates as bioindicators of water quality of effluent-receiving Ossah River, Umuahia, Southeast Nigeria*. Zanco J. Pure Appl. Sci., 31(5): 9–17, doi: http://dx.doi.org/10.21271/ZJPAS.31.5.2.

ANYANWU E.D., MBEKEE F. 2020. *Water quality and plankton assessment of Ossah River, Umuahia, Southeast Nigeria*. Taiwan Water Conserv., 68(4): 34–46, doi: 10.6937/TWC.202012/PP_68(4).0004.

ANYANWU E.D., UMEHAM S.N. 2020. *Identification of waterbody status in Nigeria using predictive index assessment tools: a case study of Eme River, Umuahia, Nigeria*. Int. J. Energ. Water Res., March 2020, https://doi.org/10.1007/s42108-020-00066-5.

APHA 1998. *Standard methods for the analysis of water and wastewater*. 21st Edition. American Public Health Association, Washington D.C.

ARIMORO F.O., ODUME O.N., UHUNOMA S.I., EDEGBENE A.O. 2015. *Anthropogenic impact on water chemistry and benthic macroinvertebrate associated changes in a southern Nigeria stream*. Environ. Monit, Assess., 187: 14, doi: 10.1007/s10661-014-4251-2.

ATOBATELE E.O., OLUTONA G.O. 2013. *Spatio-seasonal physico-chemistry of Aiba stream, Iwo, Nigeria*. Afr. J. Biotech., 12(14): 1630–1635.

ATOBATELE O.E., UGWUMBA O.A. 2008. *Seasonal variation in the physicochemistry of a small tropical reservoir (Aiba Reservoir, Iwo, Osun, Nigeria)*. Afr. J. Biotech 7: 1962–1971.

BALLOCH D., DAVIES C.E., JONES, F.H. 1976. *Biological assessment of water quality in three British rivers. The North Esk (Scotland), the Ivel (England) and the Taff (Wales)*. Water Pollut. Control., 75: 92–114.

BHATTACHARYA R.K. 2018. *In-stream sand mining impact on water quality and benthos community in an alluvial reach. A case study on River Kangsabati, West Bengal*. Int. J. Curr. Res. Life Sci., 7: 2613–2617.

Bibi F., Ali Z. 2013. *Measurement of diversity indices of avian communities at Taunsa barrage wildlife sanctuary, Pakistan*. J. Anim. Plant Sci., 23: 469–474.

Brooks A. J., Haeusler T., Reifelds I., Williams S. 2005. *Hydraulic microhabitats and distribution of macroinvertebrate assemblages in riffles.* Freshw. Biol., 50: 331–334, https://doi.org/10.1111/j.1365-2427.2004.01322.x.

Buss D.F., Baptista D.F., Nessimain J.L., Egler M. 2004. *Substrate specificity, environmental degradation and disturbance structuring of macroinvertebrate assemblages in neotropical streams. Hydrobiologia*, 518: 179–188.

Castro D.M.P., Hughes R.M., Callisto M. 2013. *Effects of flow fluctuations on the daily and seasonal drift of invertebrates in a tropical river.* Int. J. Lim., 49: 169–177, doi: 10.1051/limn/2013051.

Cobb D.G., Galloway T.D., FLANNAGAN J.F. 1992. *Effects of discharge and substrate stability on density and species composition of stream insects.* Can. J. Fish. Aquat. Sci., 49:1788–1795.

Dorak Z. 2013. *Zooplankton abundance in the lower Sakarya River Basin (Turkey). Impact of environmental variables.* J. Black Sea/Medit. Environ., 19:1–22.

Effendi H., Romanto B., Wardiatno Y. 2015. *Water quality status of Ciambulawung River Banten Province based on pollution index and NSF-WQI.* Procedia Environ. Sci., 24: 228–237.

Esenowo I.K., Ugwunba A.A.A. 2010. *Composition and abundance of macrobenthes in Majidun River, Ikorodu, Lagos state, Nigeria*. Res. J. Biol. Sci., 5: 556–560.

Etesin U., Udoinyang E., Harry T. 2013. *Seasonal variation of physicochemical parameters of water and sediments from Iko River, Nigeria.* J. Environ. Earth Sci., 3:96 – 110.

Ewa E.E., Iwara A.I., Adeyemi J.A., Eja E.I., Ajake A.O., Otu C.A. 2011. *Impact of industrial activities on water quality of Omoku Creek*. Sacha J. Environ. Stud., 1:8–16.

FMEnv. 2005. National Erosion and Flood Control Policy. Federal Ministry of Environment. Abuja, Nigeria.

FMEnv. 2011. *National Environmental (Surface and Groundwater Quality Control) Regulations*. S.I. No. 22, Gazette No. 49, Vol. 98 of 24th May, 2011. Federal Ministry of Environment, Abuja, Nigeria.

Goldschmidt T. 2016. *Water mites (Acari, Hydrachnidia): powerful but widely neglected bioindicators – a review.* Neotrop. Biodivers., 2:12–25.

Gupta S. K., Dixit S., Tiwari, S. 2005. *An assessment of heavy metals in surface waters of lower lake, Bhopal, India.* Pollut. Res., 4: 805–808.

Hammer Ø.H., Harper D.A.T., Ryan P.D. 2001. *PAST: Paleontological Statistics Software Package for Education and Data Analysis*. Palaeontol. Electron., 4(1) art. 4: 9., http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Houssou A.M., Ahouansou Montcho S., Montchowui E., Bonou C.A. 2017. *Spatial and seasonal characterization of water quality in the Ouémé River Basin (Republic of Benin, West Africa).* Egypt. J. Chem., 60: 1077–1090.

Iyagbaye L.A., Iyagbaye R.O., Omoigberale M.O. 2017. *Assessment of benthic macro-invertebrates of freshwater ecosystem: a case study of Ovia River (Iguoriakhi), Edo State, Nigeria*. Eur. Sci. J., 13: 405–422, doi: 10.19044/esj.2017.v13n26p40.

Kale V.S. 2016. *Consequence of temperature, ph, turbidity and dissolved oxygen water quality parameters.* Int. Adv. Res. J. Sci. Engr. Tech., 3:186–190.

Kalyoncu H., Gulboy H. 2009. *Benthic macroinvertebrates from Darioren and Isparta streams (Isparta/Turkey)-biotic indices and multivariate analysis*. J. Appl. Biol. Sci., 3:79–86.

Keçi E., Paparisto A., Pepa B., Xhaxhiu K. 2012. *Use of benthic macro-invertebrate taxones as biological indicators in assessing water quality of Erzeni River, Albania, During 2011–2012.* Int. J. Basic Appl. Sci., 12(6):165–169.

Koehnken L., Rintoul M.S., Goichot M., Tickner D., Loftus A.C., Acreman M.C. 2020. *Impacts of riverine sand mining on freshwater ecosystems: A review of the scientific evidence and guidance for future research.* River Res. Appl., 1–9, https://doi.org/10.1002/rra.3586.

Kucuk S. 2008. *The effect of organic pollution on Benthic macroinvertebrate fauna in the Kirmir Creek in the Sakarya Basin*. ADÜ Ziraat Fak. Derg., 5: 5–12.

LEINSTER T., COBBOLD C.A. 2012. *Measuring diversity: the importance of species similarity*. Ecology, 93: 477–489.

MAGURRAN A.E. 1988. *Ecological diversity and its measurement*. Croom Helm, London.

MANDAL S.H., DAS A., NANDA A.K. 2012. *Study of some physicochemical water quality parameters of Karola River, West Bengal an attempt to estimate pollution status*. Int. J. Environ. Protect., 2: 16–22.

MANICKAM P., SARAVANA B., SANTHANAM P., MURALISANKAR T., SRINIVASAN V., VIJAYADEVAN K., BHUVANESWARI R. 2015. *Biodiversity of freshwater zooplankton and physico-chemical parameters of Barur Lake, Krishnagiri District, Tamil Nadu, India*. Malaya J. Biosci., 2: 1–12.

MARIANTIKA L., RETNANINGDYAH C. 2014. *The change of benthic macroinvertebrate community structure due to human activity in the spring channel of the source of clouds of Singosari subdistrict, Malang Regency*. Journal Biotropika, 2: 254–259.

MASON C.F. 2002. *Biology of fresh water pollution*. (4th Edition). Pearson Educational Limited, Essex, U.K.

MCCABE D.J. 2010. *Rivers and streams: life in flowing water*. Nat. Edu. Knowl., 1(12):4

MERRITT R.W., CUMMINS K.W. 1996. *An introduction to the aquatic insects of North America*, 3rd ed., Kendall/Hunt Publishing, Dubuque, Iowa, USA.

MERRITT R.W., CUMMINS K.W., BERG M.B. 2008. *An introduction to the aquatic insects of North America*. (4thEdition). Kendall/Hunt Publishing, Dubuque, Iowa, USA.

MOHSENI O., STEFAN H.G. 1999. *Stream temperature/air temperature relationship: A physical interpretation*. J. Hydrol., 218: 128–141.

NAMAN S.M., ROSENFELD J.S., RICHARDSON J.S. 2016. *Causes and consequences of invertebrate drift in running waters: from individuals to populations and trophic fluxes*. Can. J. Fish. Aquat. Sci., 73: 1292–1305, doi.org/10.1139/cjfas-2015-0363.

NWANKWO C., NWANKWOALA H.O. 2018. *Gully erosion susceptibility mapping in Ikwuano Local Government Area of Abia State using GIS Techniques*. Earth Sci. Malay., 2(1): 08–15, doi: 10.26480/esmy.01.2018.01.07.

OBASI P.N., OKORO A.U., IGWE E.O., EDENE E.N. 2015. *Petrological characteristics and paleo-depositional environment of the sandstones of the ameki group (eocene) in Bende and Isimkpu Areas, Southeastern Nigeria*. J. Appl. Geol. Geophy., 3(5), 9–15.

OLAWUSI-PETERS O.O., AJIBARE A.O. 2014. *Species richness, diversity and abundance of some decapod crustaceans in coastal waters of Ondo State, South West, Nigeria*. Int. J. Fauna Biol. Stud., 1: 44–51.

OLDMEADOW D.F., LANCASTER J., RICE S.P. 2010. *Drift and settlement of stream insects in a complex hydraulic environment*. Freshwat. Biol., 55: 1020–1035, https://doi.org/10.1111/j.1365-2427.2009.02338.x.

RAO A.S., MARSHALL S., GUBBI J., PALANISWAMI M., SINNOTT R., PETTIGROVE V. 2013. *Design of low-cost autonomous water quality monitoring system*. International Conference on Advances in Computing, Communications and Informatics (ICACCI). Mysore, 22–25, August 2013, pp. 14–19.

REHMAN M., YOUSUF A. R., BALKHI M. H., RATHER M. I., SHAHI N., MERAJ M., HASSAN K. 2016. *Dredging induced changes in zooplankton community and water quality in Dal Lake, Kashmir, India*. Afr. J. Environ. Sci. Tech., 10(5): 141–149, doi: 10.5897/AJEST2016.2096.

SANTOS J.M., FERREIRA M.T. 2020. *Use of aquatic biota to detect ecological changes in freshwater: current status and future directions*. Water, 12: 1611, Doi: 10.3390/w12061611.

SCHEILI A., DELPLA I., SADIQ R., RODRIGUEZ M.J. 2016b. *Impact of raw water quality and climate factors on the variability of drinking water quality in small systems. Water Res. Manage.,* 30: 2703–2718, Doi: 10.1007/s1126 9-016-1312-z.

SCHEILI A., RODRIGUEZ M.J., SADIQ R. 2016a. *Impact of human operational factors on drinking water quality in small systems: an exploratory analysis*. J. Cleaner Prod., 133: 681–690. Doi: 10.1016/jjclep ro.2016.05.179.

SETIAWAN D. 2009. *The study of macrozoobenthos community at downstream waters of Lematang River surrounding in Pasar Bawah, Lahar Regency*. Sci. Res. J., 9: 12–14.

SHAH J.A., PANDIT A.K. 2013. *Application of diversity indices to crustacean community of Wular Lake, Kashmir Himalaya*. Int. J. Biodivers. Conserv., 5(6): 311–316, doi: 10.5897/IJBC2013.0567.

SHEEBA S. 2009. *Biotic environment and Sand Mining – A case study from Ithikkara River, South West Coast of India*. J. Ind. Pollut. Control, 25(2): 133–138.

SOLANKI H.A. 2012. *Status of soils and water reservoirs near industrial areas of Baroda. Pollution and soil-water Chemistry*. Lap Lambert Academic Publishing, Germany.

STUB R., APPLING J.W., HATSTETTER A.M., HASS I.J. 1970. *The effect of industrial waste of Memphis and Shelby country on primary planktonic producers*. *Bioscience*, 20: 905–912.

THORP J.H., COVICH A.P. 2001. *Ecology and classification of North American Freshwater Invertebrates*. 2nd Ed., Academic Press, San Diego, California, USA.

UHEGBU A., IGBOEKWE M.U., CHUKWU G.U., OKENGWU K.O., EKE K.T. 2013. *Aquifer characteristics and groundwater quality assessment in Ikwuano Area of Southeastern Nigeria*. J. Sci. Res. Rep., 3(2): 366–383.

UKAGWU J. I., DEEKAE S. N., OHATURUONYE S. O. 2017. *Physico-chemical studies of Akor River and distribution of prawn, Macrobrachium vollehovenii and Macrobrachium fellicinum*. Int. J. Fish. Aquat. Res., 2(3): 17–24.

UMAR D.M., HARDING J.S., MICHAEL J.W. 2013. *Freshwater invertebrates of Mambilla Plateau: photographic guide*. New Zealand: Canterbury Educational Printing Services, University of Canterbury.

WARD D., HOLMES N., JOSE P. 1995. *The new rivers and wildlife handbook*. The Royal Society for the Protection of Birds (RSPB). The Wildlife Trusts, Bedfordshire.

WEBB B.W., CLACK P.D., WALLING D.E. 2003. *Water-air temperature relationships in a Devon River system and the role of flow*. Hydrol. Process., 17: 3069–3084.

WILLOUGHBY L.G. 1976. *Freshwater biology*. Hutchinson, London.

WU L., ZHOU M., SHEN Z., CUI Y., FENG, W. 2017. *Spatio-temporal variations in zooplankton community structure and water quality in a Chinese Eutrophic River*. Appl. Ecol. Environ. Res., 15(3): 1417–1442, doi: http://dx.doi.org/10.15666/aeer/1503_14171442.

YEOM D.J., KIM J.H. 2011. *Comparative evaluation of species diversity indices in the natural deciduous forest of Mt. Jeombong*. Forest Sci. Technol., 7(2): 68–74, doi: 10.1080/21580103.2011.573940.

ZOU W., TOLONEN K.T., ZHU G., QIN B., ZHANG Y., CAO Z., KAI P., CAI Y., GONG, Z. 2019. *Catastrophic effects of sand mining on macroinvertebrates in a large shallow lake with implications for management*. Sci. Total Environ., 695: 133706, doi: 10.1016/j.scitotenv.2019.133706.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 251–270, Y. 2021

BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF *CHAMAEROPS HUMILIS* L.

***Fatima-Zahra Eddahhaoui*[1], *Chakir El Guezzane*[2], *Hicham Harhar*[3], *Abdelkader Zarrouk*[4], *Maria Boudalia*[5], *Abdelkabir Bellaouchou*[6], *Abdallah Guenbour*[7], *Mohamed Tabyaoui*[8]**

[1] ORCID: 0000-0003-0998-6139
[2] ORCID: 0000-0002-2150-1328
[3] ORCID: 0000-0001-8266-0130
[4] ORCID: 0000-0002-5495-2125
[5] ORCID: 0000-0002-1947-7517
[6] ORCID: 0000-0003-2328-2015
[7] ORCID: 0000-0001-5818-5421
[8] ORCID: 0000-0001-6556-5238
Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences
Mohammed V University in Rabat, Agdal-Rabat, Morocco

Key words: antioxidant activity, *Chamaerops humilis* L. extracts, phenolic content, phytochemical screening.

Abstract

Medicinal plants are an important source of bioactive molecules that are known for their therapeutic properties. *Chamaerops humilis* L. *(C. humilis)* among the most traditionally used therapeutic plants in Morocco, often found in the Mediterranean areas. Nowadays, medicinal plants are gradually helping to replace synthetic drugs, in order to weaken the aggressive effects of said compounds on the human body. In this study, *C. humilis* was collected in the zaer region, the extracts of the selected parts are prepared by soxhlet extraction method using methanol as solvent. Phytochemical analysis revealed the existence of tannins, flavonoids, saponins, terpenoids, and coumarins. The quantification of phenolic compound showed that the roots extract is the richest (151.09±2.83 mg GAE g^{-1} of extract), while palm heart extract is the poorest (79.12±2.17 mg GAE g^{-1} of extract). Evaluation of the antioxidant activities (AA) using the DPPH, ABTS and FRAP tests indicated that methanolic root extract has good antioxidant efficacy with IC_{50} values of 1.99±0.02 μg mL^{-1}, 37.65±0.66 μg mL^{-1}, and 279.61±4.90 μg mL^{-1}, respectively. The results of the various tests carried out are promising for a possible valorization of *C. humilis* as a bioresource in the therapeutic field.

Address: Hicham Harhar, Mohammed V University in Rabat, Av. Ibn Battouta, P.O. Box. 1014 Agdal-Rabat, Morocco, e-mail: hichamoo79@yahoo.fr

Introduction

For a long time, man has relied on the resources of nature, especially plants, for his food and medicine needs. Today, much research is being conducted on natural substances and their potential medicinal use (RHATTAS 2016 et al. 2016). It is estimated that more than 70% of medicines are derived from plant substances. Nearly 170,000 organic molecules have been identified from plants: quinine, digitaline, colchicine etc, less than half of these compounds are intended for therapeutic uses (CHAABI 2008). These organic molecules are being extensively studied for their potential use as remedies for chronic diseases such as cancer, kidney stones, ulcers and diabetes (PASSALACQUA 2006, RAMMALl 2009, SQALLI 2007). *C. humilis*, is a concrete example of a valuable natural resource because it has high ethno-pharmaceutical value (SQALLI and POLLIO 1994, BEGHALIA 2008, BENMEHDI 2012, HASNAOUI 2011). Doum is the Moroccan Arabic name of our studied plant. It belongs to the tribe of *Livistoneaes*, sub-tribe of *Raphidinaes*, of the genus *Chamaerops*, species *Chamaerops humilis* (DRANSFIELD et al. 2005). It is a floristic plant widespread in many countries of the western Mediterranean (REENÉ 1957). *C. humilis* is a well-known species since it is the only species of its family in the northern Mediterranean (QUÉZEL et al. 1999). It avoids areas that are too wet or too dry and grows spontaneously. *C. humilis* has several medicinal and non-medicinal uses. It is an ornamental plant, a source of fiber for domestic and artisanal uses (MOTTI 2009, SAVO 2013). It is also a dietary source whose heart, young shoots and fruit are edible and rich in nutrients (TARDÍO et al. 2006). Aqueous *C. humilis* leaf extract is traditionally used to treat diabetes, as it is thought to reduce total cholesterol and triglyceride levels. The traditional use of this plant suggests that it may be a potential source of medication and may be useful in the management of secondary complications of diabetes and gastrointestinal disease (GAAMOUSSI 2010). Furthermore, the fruits of *C. humilis* are used as antiseptics, while the roots are used to treat anemia and intestinal worms. These uses can be supported by the fact that the alcoholic extract of this plant has been shown to be rich in phenolic compounds that have an interesting antioxidant action (KHOUDALI 2014).

Our work is based on a comparative study between five different parts of the dwarf palm, four underground parts include the fibers, bark, roots and heart of the palm, and the other aerial part includes the leaves.

Materials and Methods

Preparation of extracts

The studied parts of C. *humilis* are collected in the Zaer region (75.9 km to Rabat, Morocco) in December 2018, the different parts dried for about ten days in the shade. 15 g of mixed material from each sample are placed in a cellulose cartridge and subjected to extraction by the soxhlet for 6 hours with hexane, followed by a second extraction with methanol for 4 hours. The extracts are then concentrated under vacuum using a rotary vacuum evaporator (Heidolph G1, Germany) at 60–75°C and the dried residues are stored at -4°C for further studies.

Moisture determination

Moisture is the amount of water contained in plant matter. This parameter was measured by the oven drying method at 105±5°C. To ensure good preservation, the moisture content must be less than or equal to 10% (SHARIFIFAR et al. 2007). The percentage of moisture is calculated by the following formula:

$$M\,[\%] = \frac{(m_1 - m_2)}{m_1} \cdot 100$$

where:
M – percentage of moisture
m_1 – weight of the sample in grams after harvesting of the fresh plant.
m_2 – weight of sample in grams after drying (dried plant).

Phytochemical screening

Phytochemical screening is a qualitative test based on precipitation and turbidity reactions or color change. These reactions are used to determine the presence or absence of secondary metabolites in the *C. humilis* parts.

We characterized the different chemical groups using methanolic extracts following the techniques as described in published literature (CHATOUI 2016, LABIAD 2017).

Alkaloids

Alkaloids are detected in extracts with the Mayer reagent by a precipitation reaction. Each methanolic extract was dissolved in a few mL of 50% hydrochloric acid, the formation of a yellow precipitate after the addition of drops of reagent indicates the presence of alkaloid (ABBAS 2014).

Tannins

1.5 g of each dry extract is mixed with 10 mL of methanol (80%), and stirred for 15 minutes; the extracts are filtered and placed in test tubes. The presence or absence of tannins is detected by the addition of 1% $FeCl_3$. The blue-black and green-brown color indicates the presence of gallic and catechic tannins respectively (DOHOU et al. 2003).

Flavonoids

The detection of flavonoids is based on the reaction of cyanidin. Two mL of extract are vaporized, and the residue is absorbed in 5 mL of HCl-EtOH (2:1, v/v). 2 to 3 parts of magnesium are added to cause heat release, which subsequently results in pink or purplish coloration. This coloration intensifies after the addition of 3 drops of isoamyl alcohol, which indicates a positive reaction (AZZI 2013).

According to Liebermann's reaction, 5 mL of each extract is vaporized on a sand bath. The residue is dissolved hot in 1 mL of acetic anhydride. The addition of 0.5 mL of concentrated sulphuric acid produces a purple color which changes to blue and then green indicating the existence of polyterpenes and sterols (YAM et al. 2009).

Coumarins

In a test tube, 0.5 mL of sodium hydroxide solution (10%) is added to 2 mL of the extract dissolved in methanol, and heated to boiling. Then 4 mL of pure water are added to the mixture. When the tube becomes transparent, this reflects the presence of coumarins.

Saponins

The mixture of 1 mL of the extract with two mL of hot pure water was stirred vigorously for 15 seconds and then left to stand for a quarter-hour. Stable and persistent foam greater than one centimeter in height indicates the presence of saponins (BEKRO 2007).

Starch

10 mL of a saturated solution of NaCl are added to the aqueous extract (1 mL). After heating, starch is added as a reagent, if the mixture turns a purplish blue, it indicates the presence of starch.

Protein

Proteins are detected in the residues of certain parts of *C. humilis* by the biuret reaction. A few milligrams of each residue are dissolved in two mL of the aqueous solution of NaOH 20%, then 2 to 3 drops of a 20% aqueous solution of $CuSO_4$. The appearance of a purple color, sometimes with a reddish tinge, indicates a positive reaction.

Determination of secondary metabolites

Total phenolic content (TPC)

The determination of polyphenols content of methanolic extracts was carried by UV-Visible spectrophotometry using the Folin Ciocalteu reagent (F-C) method (LISTER 2001). In a test tubes, 0.5 mL of sample solution was mixed with 0.5 mL of the aqueous solution of Folin (10%) and 4 mL of Na_2CO_3 (7.5%). The tubes were placed for 30 minutes in a water bath at 45°C. The absorbance was measured at 765 nm. TPC was estimated using a standard gallic acid reagent curve; results were expressed as milligram gallic acid equivalent per gram of extract (mg GAE g^{-1} E).

Total flavonoids content (TFC)

The aluminum trichloride ($AlCl_3$) method is used to determine the flavonoids content (RIAZ et al. 2009). The test consisted in adding 1 mL of sample solution prepared in methanol with 6.4 mL of pure water and 0.3 mL of $NaNO_2$ 5%, after 5 minutes, 0.3 mL of $AlCl_3$ solution at 10% was added, the whole was left for 6 minutes before adding 2 mL of NaOH solution (1 M). The absorbance was measured at 510 nm after 30 minutes of incubation. The TFC was calculated using a standard quercetin curve (mg QE g^{-1} E).

Total tanins content (TTC)

The vanillin acid method consists of determining the condensed tannin content of each extract (SUN 1998). The assay consists of mixing 100 µL of the methanolic solution of the sample with 3 mL of the vanillin solution prepared in methanol (4%), 1.5 mL of hydrochloric acid (HCl) (37%) is added to the mixture, the latter is put 20 minutes in the dark, the absorbance was read at 500 nm by spectrophotometer. The results were expressed as milligram of catechin equivalent per gram of extract (mg CE g^{-1} E).

Antioxidant capacity

Radical scavenging activity of 1,1-diphenyl picryl hydrazyl (DPPH)

The DPPH• test measures the antioxidant power of plant extracts using an organic solvent. This test consists in reducing the free radical DPPH• by hydrogen transfer, the reaction involved causes a transformation of the blue color of the DPPH solution to pale yellow. Briefly, 0.5 mL of the 0.2 mM methanolic DPPH solution was added to 2.5 mL of the stock extract solution at different concentrations and standards (Ascorbic Acid and Trolox). The solution of each sample is then incubated for 30 min in the dark, the absorbance was measured at 517 nm compared to blank samples (El Moudden et al. 2019).

ABTS radical scavenging test

The following method is used to reduce the cationic radical ABTS $^{+\bullet}$. This method is described by Arnao (2001). The procedure consists in preparing a solution of 2 mM ABTS and 70 mM potassium persulfate ($K_2S_2O_8$) in equal volumes and leave it stirred in the dark for 12 to 16 hours at ambient temperature. The solution obtained should be read at an absorbance of (0.70±0.02) at 734 nm by dilution in methanol. 2 mL of the prepared solution was added to 200 µL of the methanolic solution of the extracts and the standard (Trolox) at different concentrations, the absorbance was known at 734 nm after 30 min. The difference in absorbance between the ABTS solution in the presence and absence of the sample for the observation of the potential of the compounds responsible for this activity to reduce this radical.

Calculation of the antioxidant activity

The antioxidant capacity by two methods DPPH and ABTS was then calculated using the following formula:

$$\text{AA [\%]} = \frac{(\text{Abs } C - \text{Abs } E)}{\text{Abs } C} \cdot 100$$

where:
Abs C – control absorbance
Abs E – absorbance of the extract.

The inhibitory concentration (IC_{50}) was determined as the concentration of the extract which produces 50% of the trapping effect of the free radicals (DPPH and ABTS).

Reducing power activity (FRAP)

The reduction reaction of ferric ions to ferrous ions is a method determined and modified by TOPÇU et al (2007). It consists of mixing 1 mL of stock solution of the studied extract and standard (ascorbic acid) at different concentrations with 2.5 mL of a 0.2 M phosphate buffer solution at pH = 6.6, and 2.5 mL of a potassium ferricyanide solution $K_3Fe(CN)_6$ (1% w/v). The mixture is incubated for 20 minutes in a water bath at 50°C. The reaction is then stopped by adding 2.5 mL of trichloroacetic acid TCA (10% w/v). The tubes are put in the centrifuge for 10 min at 3000 rpm. 2.5 mL of the supernatant is collected and mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous solution of ferric chloride ($FeCl_3 \cdot 6H_2O$). The reading of the absorbance is done at 700 nm against a blank prepared in the same manner by pure water.

The effective concentration (EC_{50}) was defined at absorbance 0.5 of the graph. This parameter allows to compare and identify the reducing capacity of the bioactive molecules contained in each extract studied.

Data analysis

The analysis of variance ANOVA was realized by the statistical analysis software IBM SPSS Statistics 21, to validate the statistical significance by Tukey's test at 95.0% confidence level, The values are given as of triplets means ± standard error of the mean. The correlation between all the results of the variables of the studied extracts of *C. humilis* was performed by Pearson's correlation. As well as the combination of the variables with the extracts of this study was done by PCA in the form of a graphical representation, and that of HCA was done to pursue the relationships between all the samples in clusters based on the characteristics of the bioactive agents measured. These three multivariate statistical treatments are performed by XLSTAT 2014 software (ZIELINSKI 2014).

Results and Discussion

Moisture content and extracts yields determination

The results of moisture content are presented in Table 1 and indicate a variation from 29.56±1.48 to 68.96±3.31%, in the different part of the *C. humilis*. There is no significant difference in the moisture content of fibres, barks and roots, however palm hart records the high moisture content, while leaves have a moisture content of 50.10±3.33% (Table 1). These values suggest short-term storage.

Table 1

Moisture and Extracts yields of different parts of *C. humilis*

Specification	Moisture content [%]	Extract yield	
		hexanic extract [%]	methanolic extract [%]
Fibers	34.66±1.98^{a}	3.11±0.34^{a}	12.78±1.30^{a}
Barks	31.71±1.34^{a}	2.65±0.32^{ba}	25.24±2.48^{b}
Roots	29.56±1.48^{a}	2 .51±0.12^{ab}	15.70±2.21^{ac}
Palm heart	68.96±3.31^{b}	1.12±0.08^{c}	16.78±1.10^{abc}
Leaves	50.11±3.33^{c}	1.63±0.07^{bc}	22.98±1.42^{bc}

The means are presented as triplicate (n = 3e±SEM), values followed by the same letters in the same column are not different ($P < 0.05$)

The extraction method must allow the extraction of a maximum of phenolic compounds without alteration (Hayouni 2007). The solubility of phenolic compounds is governed by their nature, by the extraction method chosen and by the polarity of the solvents used. (Goli 2005, Roby 2013, Sulaiman et al. 2011).

Table 1 showed that there was a significant difference between the extraction rate according to the part of the plant and the solvent used. A high yield of extract was found in the solvent with high polarity (methanol). While hexane that extracting lipid fraction was in low yield. The same results were found by Goli et al. (2005) for pistachio, the extraction rate increases according to the polarity of the solvent. Methanol is a solvent of high polarity, which explains the relatively high total polyphenol contents recorded in methanolic extracts (Roby et al. 2013).

Phytochemical screening

Test samples were screened phytochemically for coumarins, proteins, alkaloids, tannins, flavonoids, starches, saponins and terpenoids. The results are shown in Table 2.

Table 2

Phytochemical screening of different parts of *C. humilis*

Specification	Fibers	Barks	Roots	Palm heart	Leaves
Coumarins	+	+	+	++	+
Proteins	-	-	-	-	-
Alkaloids	-	-	-	-	-
Catechic tannins	++	+++	+	-	+++
Gallic tannins	+	+	+	-	++
Flavonoids	++	++	+++	+	++
Starchs	-	-	-	-	-
Saponins	++	++	+++	++	++
Sterols and polyterpenes	+	+	+	++	+++

The results are interpreted as follows: (+) weak presence, (++) medium presence, (+++) strong presence and (-) absence

The phytochemical evaluation of the methanolic extracts of the five parts of *C. humilis* shows that it contains: tannins, coumarins, saponisides, flavonoids, and terpenes. The presence of the metabolites present in each part of the plant differs according to the degree of coloration or the quantity of the precipitate.

Phytochemical tests carried out on the methanolic extract of C. *humilis* swarm leaves confirmed the presence of flavonoids, tannins, saponins, terpenoids, and the absence of steroids (BENMESSAOUD et al. 2018). The results obtained are similar to those reported previously in Algeria (Tlemcen region) and Morocco (Benslimane region) (BENMEHDI et al. 2012, KHOUDALI et al. 2014). This means that the origin has no influence on the presence of the chemical families, but the content of each metabolite may vary from one region to another. In addition, extracts of C. *humilis* roots from Algeria indicated the presence of phenolic compounds, flavonoids, quinons, tannins, saponins and coumarins in the leaflet (BENAHMED-BOUHAFSOUN 2013).

Since C. *humilis* contains flavonoid, it is likely to have antitumor, anticarcinogenic, anti-inflammatory, hypotensive and diuretic activities (JEAN 2009). C. *humilis* contains also coumarins, which have different effects on plant development depending on their concentration and also depending on the species. They are considered phytoalexins, the metabolites that the plant synthesizes in large quantities to fight infections caused by fungi or bacteria and they also have antiedematous and vasoprotective activities (HOFFMANN 2003).

It should also be mentioned that the chemical family of tannins, found also in C. *humilis,* has antiviral, antibacterial and anti-tumor activity. It has also been reported that some tannins are used as diuretics (EZEABARA et al. 2014). Saponins, which have been detected in the extract of parts of C. *humilis,* are responsible for many pharmacological properties, such as inhibitory effects on inflammation (ESTRADA 2000, JUST et al. 1998).

Total phenolic, flavonoid and tannin content (TPC, TFC, and TTC)

Phenolic compounds are active principles responsible for antioxidant powers in biological systems, by dint of its redox properties, which can play an important role in the neutralization of free radicals by absorption, the decomposition of peroxides, or the deactivation of singlet and triplet oxygen (ARORA and CHANDRA 2011). The quantitative study of our different extracts aims to determine total phenolic content: TPC, total flavonoid content: TFC and total tannins content: TTC.

The total phenolic content was varied from 79.12±2.17 to 151.09±2.83 mg EAG g^{-1} extract. The root is the richest part of phenolic compounds. No significant difference ($p > 0.05$) between fibers, bark and leaves extracts were observed, while palm heart extracts had lower values (79.12±2.17 mg GAE g^{-1} E). The results obtained were lower than those reported by BENMESSAOUD et al. (2018), for the leaves (125.847 mg GAE g^{-1} E). While the results of BENAHMED-BOUHAFSOUN et al. (2013) showed lower total content in roots and leaves with values of 26 to 28.7 mg GAE.g^{-1} of extract, respectively.

Similarly, the roots have the highest flavonoid content, with 50.81±2.5 mg QE g^{-1} of extract, followed by the three parts, bark, fibers and leaves, which are partially of the same order (Table 3). Moreover, the palm heart

Table 3

Total content of phenols, flavonoids and tannins in different parts of *C. humilis* extracts

Specification	TPC [mg GAE g^{-1} E]	TFC [mg QE g^{-1} E]	TTC [mg CE g^{-1} E]
Fibers	108.86±4.54^{a}	34.18±2.1^{a}	8.86±0.73^{a}
Barks	122.01±6.91^{a}	36.91±2.75^{a}	10.07±1.21^{a}
Root	151.09±2.83^{b}	50.81±2.5^{b}	7.83±0.35^{a}
Palm heart	79.12±2.17^{c}	19.35±1.56^{c}	–
Leaves	97.74±3.95^{a}	39.12±2.5^{ab}	1.97±0.69^{b}

The values are given as of triplets mean ± SEM, with similar letters in the different columns indicating significant ($P < 0.05$). GAE – gallic acid equivalent; QE – quercetin equivalent; CE – catechin equivalent; TPC – total phenolic content; TFC – total flavonoid content; TTC – total tannin content had the lowest flavonoid content (19.35±1.56 mg QE per 1 g of extract). BENAHMED-BOUHAFSOUN (2018) found that flavonoid content of the leaves is twice as high as that of the roots, with respective contents of 40.7±0.53, and 20.04±0.62 mg EQ g^{-1} of extract, and reported a low content of flavonoid in the leaves (2.663±0.244 mg QE g^{-1} of extract)

As regards the tannin content, all parts are poor, with a value varying between 10.07±1.71, and 1.97±0.98 mg CE g^{-1} E, for the palm heart the amount of tannin was not detected. DJIPA et al. (2000) reported that the fibers contain 0.37 % of tannins

Antioxidant activity

The IC_{50} is a parameter that defines the concentration of antioxidants needed to reduce the initial concentrations of free radicals by 50%. The lower the IC_{50} value the greater the antioxidant action. The IC_{50} values of the alcoholic extracts, as well as those of the standards are shown in Table 4.

Table 4

IC_{50} results of different parts of *C. humilis* methanolic extracts and standards

Specification	DPPH IC_{50} [μg mL^{-1}]	ABTS IC_{50} [μg mL^{-1}]	FRAP EC_{50} [μg mL^{-1}]
Fibers	$4.15±0.02^a$	$68.87±3.09^a$	$448.27±8.73^a$
Barks	$3.95±0.71^a$	$41.82±2.47^b$	$292.52±6.89^b$
Roots	$1.99±0.02^a$	$37.65±0.66^b$	$279.61±4.90^b$
Palm heart	$36.94±0.9^b$	$485.6±12.03^c$	$2315±10^c$
Leaves	$7.015±0.66^c$	$98.03±2.47^d$	$599.94±7.56^d$
Ascorbic acid	$1.91±0.04^a$	–	$45.8 ±1.9^e$
Trolox	$2.96±0.8^a$	$30.86±0.04^b$	–

Data are presented as the mean of triplicates ± SEM, with numbers followed by similar letters in the same column not different with $P < 0.05$

Methanolic extracts of *C. humilis* fibers, bark, roots, leaves and heart of palm reduce free radicals with respective IC_{50} ranging from 1.99 to 36.94 μg mL^{-1} for DPPH and from 4.24 to 64.02 μg mL^{-1} for the cationic radical ABTS. The values follow the same order of predominance of the different parts studied. In addition, the roots have a very high activity which is practically similar to that of ascorbic acid which brings stability to DPPH with an IC_{50} of 1.91±0.04 μg mL^{-1}, this part has an IC_{50} equal to 1.99±0.02 μg mL^{-1} which is in part low compared to that of trolox 2.96±0.8 μg mL^{-1}. Similarly for the neutralization of ABTS, the roots and barks retains the same IC_{50} order of 37.65±0.66 µg mL^{-1}, and 41.82±2.47 µg mL^{-1}, respectively. These values do not have a significant difference with that of the trolox which has an IC_{50} of 30.86±0.04 µg mL^{-1}, followed by fibers, which also have a remarkable value equal to 68.87±3.09 µg mL^{-1}.

Roots and barks have a capacity practically close to the reduction of ferric ions to ferrous ions with an EC_{50} of 279.61±4.90 and 292.52±6.89 µg mL^{-1}, respectively, then the fibers and finally the leaves which show a statistically significant difference, the latter two parts have a mean reduction compared to the others, but not the heart of the palm. According to these results, it is proven that ascorbic acid remains the most effective reducing agent with an EC_{50} of 45.8±1.9 µg mL^{-1} compared to the methanolic extracts of the different parts of *C. humilis* studied.

The results of the three antioxidant tests carried out show that the underground part has the best antioxidant activity, and the primary roots (the part which gathers the fibers, the barks and the heart of the stipe) are the most effectively used with low concentrations.

The polyphenols contained in the extracts obtained are probably responsible for the antioxidant activity. It can be noted that all the parts keep their order of predominance, the primary root has the highest levels of secondary metabolites, and this reflects the fact that it has the lowest IC_{50}, followed by bark, fibers and leaves. Nevertheless, the heart of the palm is the part that contains the lowest levels of active ingredients, reflecting its low reducing activity.

The results of the IC_{50} of the methanolic extract of the leaves show good antioxidant activity for DPPH, ABTS and FRAP with IC_{50} of 346.08±12.63 µg mL^{-1}, 593.23±9.80 µmol TE g^{-1} and 434.34±13.71 µmol TE g^{-1} extract, respectively, compared to the standard using BHT with an IC_{50} value of 462.24±0.03 µg mL^{-1} (Gonçalves et al. 2018).

The Antioxidant activity of *C. humilis* from leaves collected in Algeria, has already been tested by Benahmed (2013). The IC_{50} results reported IC_{50} of about 180.71±6.6 µg mL^{-1} in a DPPH test, comparing to the ascorbic acid 159.5±4.81 µg mL^{-1}.

Correlation Matrix

Table 5 shows the Pearson correlation, which allows us to analyze the relationship between the different variables tested in this study. Thus, Table 6 shows the *p*-values of the coefficients of the correlation matrix between all the variables. Based on the results obtained in the Table 5 and Table 6, we observed a significant positive correlation (p-value < 0.05) between TFC and TPC ($r^2 = 0.898$). In addition, a highly significant positive correlation (p-value < 0.05) was detected between TPC and DPPH ($r^2 = 0.987$), TPC and ABTS ($r^2 = 0.956$), and FRAP ($r^2 = 0.936$), respectively. This indicates that the phenolic content of our extracts contributes to its ability to donate electrons to hydrogen. This reflects that the antioxidant capacity of our samples can be attributed to the existence of total phenols.

Table 5

Coefficient of the Pearson correlation matrix between the variables: TFC, TTC, TPC, ABTS, DPPH and FRAP of the different parts of *C. humilis*

Variables	TPC	TFC	TTC	($1/IC_{50}$) to DPPH	($1/IC_{50}$) to ABTS	($1/IC_{50}$) to FRAP
TPC	**1**	–	–	–	–	–
TFC	0.898	1	–	–	–	–
TTC	0.740	0.559	**1**	–	–	–
($1/IC_{50}$) to DPPH	**0.987**	**0.899**	0.699	**1**	–	–
($1/IC_{50}$) to ABTS	**0.956**	0.839	0.856	**0.905**	**1**	–
($1/IC_{50}$) to FRAP	**0.936**	0.837	0.877	**0.883**	**0.997**	**1**

Values in bold in Table 5 have a significant correlation at the level: alpha = 0.05

Table 6

p-values of the correlation matrix coefficient between all variables

Variables	TPC	TFC	TTC	($1/IC_{50}$) to DPPH	($1/IC_{50}$) to ABTS	($1/IC_{50}$) to FRAP
TPC	**0**	–	–	–	–	–
TFC	**0.038**	0	–	–	–	–
TTC	0.153	0.327	**0**	–	–	–
($1/IC_{50}$) to DPPH	**0.002**	**0.038**	0.189	**0**	–	–
($1/IC_{50}$) to ABTS	**0.011**	0.075	0.064	**0.034**	**0**	–
($1/IC_{50}$) to FRAP	**0.019**	0.077	0.051	**0.047**	**0.000**	**0**

Values in bold in Table 6 have a significant correlation at the level: alpha = 0.05

The positive linear correlation between the phenolic content and the antiradical capacity has also been reported by several authors (GUETTAF et al. 2016, AMRI et al. 2015). There was also a significant ($p < 0.05$) positive correlation between TFC and DPPH ($r^2 = 0.899$). However, tannins do not contribute much to the antioxidant activities tested; hence, its correlation coefficients are relatively low. Contrary to polyphenols and flavonoids which contribute strongly to this bioactivity. Therefore, these strong correlations indicate that the bioactive agents, which contribute to the iron-reducing power by FRAP, are themselves the source of the free radical scavenging power by DPPH and ABTS assays. Furthermore, the correlation matrix showed a strong correlation between the three antioxidant tests, between DPPH and ABTS (r^2 = 0.905), and between DPPH and FRAP (r^2 = 0.883), and between FRAP and ABTS (r^2 = 0.997) with (p-value < 0.05). The positive correlation between the three antioxidant tests is hard to the strong presence of total phenols, the results found are very promising, and this allows us to say that our samples, as well as our chosen plant can be considered from the best natural antioxidants.

Principal Component Analysis (PCA)

The results of polyphenol, flavonoid, and tannin contents, and the antioxidant activity DPPH, ABTS and FRAP are considered as variables. They are projected by PCA on the F1–F2 factorial planes (Figure 1).

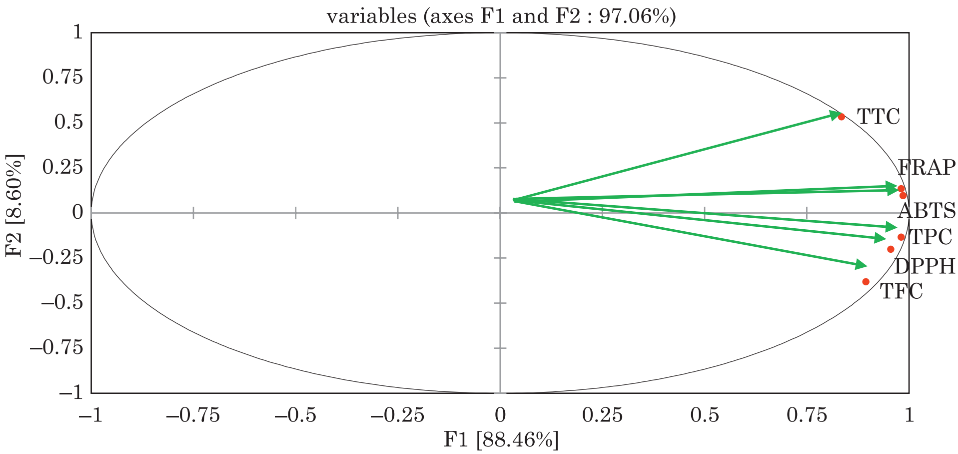


Fig. 1. Correlation circles between all variables

The first major component F1 explains 88.46% of the total information and the second major component F2 presents 8.60%. The cumulative percentage of the first two major components being 97.06 %, its linear combination represents the variables, as it is greater than 50%. Therefore, the first two axes are appropriate to explain the information as a whole. Figure 1 shows the plane formed by the F1 and F2 axes, which gives the correlation between the variables. The F1 and F2 axes consist mainly of the positive correlation between TTC, TFC, TPC, and between DPPH, ABTS and FRAP tests.

The theoretical data in Figure 2 show the existence of three groups. The first group is formed by an extract called roots, which is characterized by the highest contents of total polyphenols and flavonoids, as well as by its very high antioxidant efficiency by DPPH, ABTS and FRAP.

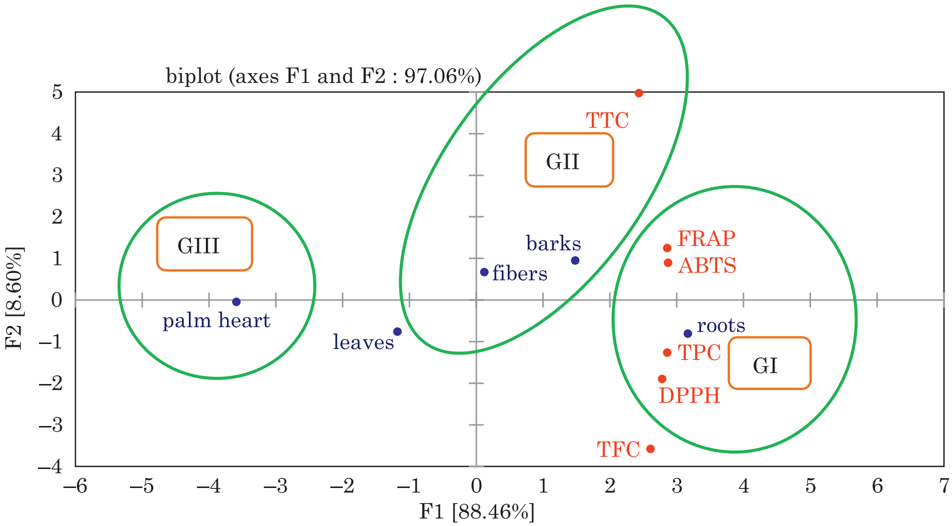


Fig. 2. Correlation variables-parts (GI – group I; GII – group II; GIII – group III)

The second group consists of bark, fiber and leaves, which are characterized by a higher TTC value. In addition, they have average levels of TPC and TFC. Likewise, their antiradical activities (DPPH, ABTS and FRAP) are close to those of the first group. It is also observed that the roots have an average TTC content. The rest of the studied part (heart palm) belongs to the 3rd group, which is far from the other two groups, reflecting that the heart palm has very low contents of the studied secondary metabolites compared to the other parts. Thus, their tested inhibitory actions are also low.

The analysis of the data by PCA, showed the presence of a very high positive correlation, between phenolic compounds and antioxidant activity by DPPH, ABTS and FRAP. This showed that in our extracts, the bioactive compounds, which provide the free radical scavenging activity, as well as the reduction of ferric ions to ferrous ions, by DPPH, ABTS and FRAP tests, respectively.

Hierarchical clustering analysis (HCA)

According to HCA, the extracts were grouped by Euclidean squared method and Wards method to identify the (dis)similarity measure. The HCA was used to evaluate the correlation between the extracts, as well as to present the similarities of the 5 tested samples based on the data of bioactive agent contents and antioxidant activity, as shown in the dendrogram in Figure 3. According to the bioactive molecules, the 5 extracts were classified into three clusters.

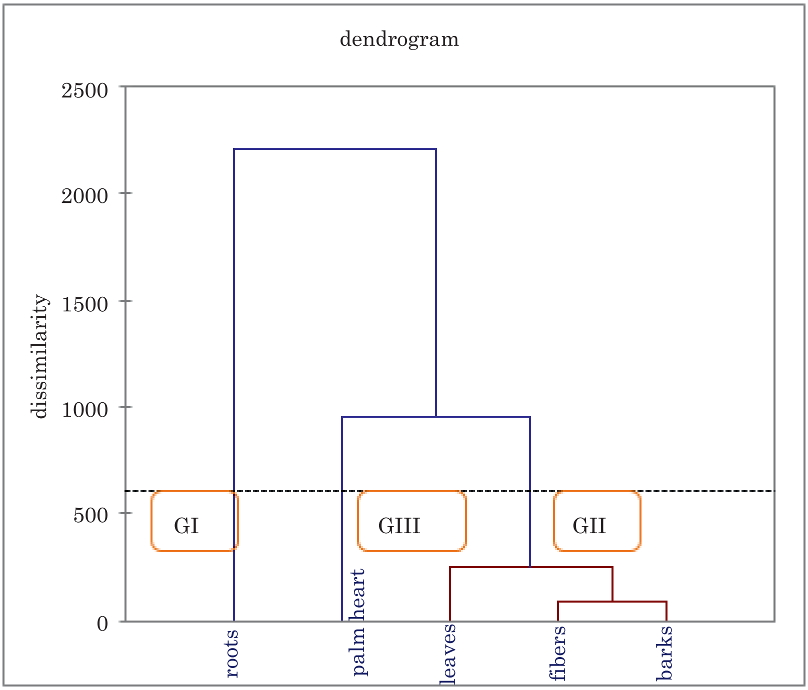


Fig. 3. Dendrogram of the extracts tested founded by HCA, using on bioactive and their antioxidant capacities (GI – cluster I; GII – cluster II; GIII – cluster III)

Cluster II, contains a single extract of the roots, and which represented 20% of the total extracts. This extract had the highest value of TPC, TFC, and TTC reached about: 151.09±2.83 mg GAE g^{-1} E, 50.81±2.5 mg QE g^{-1} E, and 7.83±0.35 mg CE g^{-1} E, respectively. This allows it to be characterized by a strong antioxidant power by DPPH, ABTS and FRAP tests.

Cluster II, is formed by 3 extracts named respectively leaves, fibers, and barks, representing 60% of the total extracts, characterized by a medium mean values of TPC, TFC, and TTC (see Table 3), as well as by an average antioxidant capacity compared to that of cluster I.

Cluster III contained an extract, called palm heart, representing 20% of the total extracts, characterized by low values of secondary metabolites, TPC (79.12±2.17 mg GAE g^{-1} extract), and TFC (19.35±1.56 mg QE g^{-1} extract), respectively. As well as lower antioxidant power. These results are consistent with the data obtained by PCA, in which the distribution of all extracts on the score plot indicates an identical trend.

Conclusion

Phytochemical tests performed on the five parts reveal the existence of interesting chemical families: tannins, flavonoids, saponins, coumarins and terpenes. The quantitative analyses carried out show that the methanolic extracts of the roots, barks, fibers and leaves of *C. humilis* are rich in phenols, flavonoids and tannins. Given the results obtained in this study, it can be concluded that the three methods used can quantify the antioxidant activity of the 5 part of the plant species, this activity can be attributed to the high existence of phenolic compounds. The results of the various tests carried out are promising for a possible valorization of *C. humilis* as a bioresource in the therapeutic field.

Conflicts of Interest

Authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors would thank Dr H El Moudden and Y. Halabi for their helpful discussion along with the realization of this work.

References

Abbas M., Shahid M., A. Sheikh M., Muhammad G. 2014. *Phytochemical screening of plants used in folkloric medicine. Effect of extraction method and solvent*. Asian J. Chem., 6(18): 6194–6198, http://dx.doi.org/10.14233/ajchem.2014.17125.

Aliotta G., Pollio A. 1994. *Useful plants in renal therapy according to Pliny the Elder*. Am. J. Nephrol., 14(4–6): 399–411, https://doi.org/10.1159/000168755.

Arnao M.B., Cano A., Acosta M. 2001. *The hydrophilic and lipophilic contribution to total antioxidant activity*. Food Chem., 73(2): 239–244.

Amri O., Elguiche R., Tahrouch S., Zekhnini A., Hatimi A. 2015. *Antifungal and antioxidant activities of some aromatic and medicinal plants from the southwest of Morocco*. J. Chem. Pharm. Res., 7(7): 672–678.

Arora D.S., Chandra P. 2011. *Antioxidant activity of Aspergillus fumigatus*. ISRN Pharmacol., 1–11, https://dx.doi.org/10.5402/2011/619395.

Azzi R. 2013. *Contribution à l'étude de plantes médicinales utilisées dans le traitement traditionnel du diabète sucré dans l'Ouest algérien: enquête ethnopharmacologique; Analyse pharmaco-toxicologique de Figuier (Ficus carica) et de coloquinte (Citrullus colocynthis) chez le rat Wistar* (doctoral dissertation).

Beghalia M., Ghalem S., Allali H., Belouatek A., Marouf A. 2008. *Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants*. Res. J. Medicinal Plant., 2(3): 66–70.

Bekro Y.-A., Mamyrbekova J.A., Boua B.B., Bi F.H.T., Ehile E.E. 2007. *Etude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. et Zarucchi (Caesalpiniaceae).*, Sci. Nat, 4(2): 217–225.

Benahmed-Bouhafsoun A., Djied S., Mouzaz F., Kaid-Harche M. 2013. *Phytochemical composition and in vitro antioxidant activity of Chamaerops humilis* L. *extracts*. Int. J. Pharm. Pharm. Sci., 5: 741–744.

Benmehdi H., Hasnaoui O., Benali O., Salhi F. 2012. *Phytochemical investigation of leaves and fruits extracts of Chamaerops humilis* L. J. Mater Environ Sci., 3: 320–337.

Benmessaoud Left D., Zertoubi M., Khoudali S., Azzi M. 2018. *New Application of Chamaerops Humilis* L. *extract as a green corrosion inhibitor for reinforcement steel in a simulated carbonated concrete pore solution*. Port. Electrochimica Acta., 36(4): 249–257, http://dx.doi.org/10.4152/pea.201804249.

Chaabi M. 2008. *Étude phytochimique et biologique d'espèces végétales africaines: Euphorbia stenoclada Baill. (Euphorbiaceae), Anogeissus leiocarpus Guill. & Perr.(Combretaceae), Limoniastrum feei (Girard) Batt.(Plumbaginaceae).*

Chatoui K., Talbaoui A., Aneb M., Bakri Y., Harhar H., Tabyaoui, M. 2016. *Phytochemical screening, antioxidant and antibacterial activity of Lepidium sativum seeds from Morocco*. J. Mater. Environ. Sci., 7(8): 2938–2946.

Djipa C.D., Delmée M., Quetin-Leclercq J. 2000. *Antimicrobial activity of bark extracts of Syzygium jambos (L.) Alston (Myrtaceae).* J. Ethnopharmacol., 71(1–2): 307–313, https://doi.org/10.1016/S0378-8741(99)00186-5.

Dohou R., Yamni K., Tahrouch S., Hassani L.M.I., Badoc A., Gmira N. 2003. *Screening phytochimique d'une endémique iberomarocaine, Thymelaea lythroides*. Bulletin-Société de Pharmacie de Bordeaux., 142(1/4): 61–78.

Dransfield J., Uhl N.W., Asmussen C.B., Baker W.J., Harley M.M., Lewis C.E. 2005. *A new phylogenetic classification of the palm family, Arecaceae*. Kew Bull., 60: 559–569.

El-Guezzane C., El-Moudden H., Harhar H., Chahboun N., Tabyaoui M., Zarrouk A. 2021. *A comparative study of the antioxidant activity of two Moroccan prickly pear cultivars collected in different regions*. Chem Data Collect., 31: 100637, https://doi.org/10.1016/j.cdc.2020.100637.

El Moudden H., El Idrissi Y., El Yadini A., Harhar H., Tabyaoui B., Tabyaoui M. 2019. *Effect of filtration of olive mill wastewater on the phenolic composition and its influence on antioxidant activity*. Pharmacologyonline, 2(162): 161–176.

Estrada A., Katselis G.S., Laarveld B., Barl B. 2000. *Isolation and evaluation of immunological adjuvant activities of saponins from Polygala senega L.* Comp. Immunol. Microbiol. Infect. Dis., 23(1): 27–43, https://doi.org/10.1016/S0147-9571(99)00020-X.

Ezeabara C.A., Okeke C.U., Ilodibia C.V., Aziagba B.O. 2014. *Determination of tannin content in various parts of six citrus species*. J. Sci. Rep., 3: 1384–1392, https://doi.org/10.9734/JSRR/2014/5832.

Gaamoussi F., Israili Z.H., Lyoussi B. 2010. *Hypoglycemic and hypolipidemic effects of an aqueous extract of Chamaerops humilis leaves in obese, hyperglycemic and hyperlipidemic Meriones shawi rats*. Pak. J. Pharm. Sci., 23(2): 212–9.

Guettaf S., Abidli N., Kariche S., Bellebcir L., Bouriche H. 2016. *Phytochemical screening and antioxidant activity of aqueous extract of Genista Saharae (Coss. &Dur.).* Der Pharm. Lett., 8(1): 50–60.

GOLI A.H., BARZEGAR M., SAHARI M.A. 2005. *Antioxidant activity and total phenolic compounds of pistachio (Pistachia vera) hull extracts*. Food chem., 92(3): 521–525, https://doi.org/10.1016/j.foodchem.2004.08.020.

GONÇALVES S., MEDRONHO J., MOREIRA E., GROSSO C., ANDRADE P.B., VALENTÃO P., ROMANO A. 2018. *Bioactive properties of Chamaerops humilis* L.*: antioxidant and enzyme inhibiting activities of extracts from leaves, seeds, pulp and peel*. 3 Biotech., 8(2): 88.

HASNAOUI O., BOUAZZA M., BENALI O., THINON M. 2011. *Ethno botanic study of Chamaerops humilis* L. *var. argentea Andre (Arecaceae) in Western Algeria*. Agric. J., 6(1): 1–6, http://dx.doi.org/10.3923/aj.2011.1.6.

HAYOUNI E.A., ABEDRABBA M., BOUIX M., HAMDI M. 2007. *The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus coccifera* L. *and Juniperus phoenicea* L. *fruit extracts*. Food Chem., 105(3): 1126–1134.

HOFFMANN L. 2003. *Etude du métabolisme des phénylpropanoïdes; analyse de l'interaction de la caféoyl-coenzyme A 3-O-méthyltransférase (CCoAOMT) avec son substrat et caractérisation fonctionnelle d'une nouvelle acyltransférase, l'HydroxyCinnamoyl-CoA: shikimate/quinate hydroxycinnamoyl Transférase (HCT)* (thesis).

JEAN B. 2009. *Pharmacognosie, phytochimie, plantes médicinales (4e éd.)*. Lavoisier.

JUST M.J., RECIO M.C., GINER R.M., CUÉLLAR M.J., MÁÑEZ S., BILIA A.R., RÍOS J.L. 1998. *Anti-inflammatory activity of unusual lupane saponins from bupleurum fruticescens*. Planta Med., 64(05): 404–407.

KHOUDALI S., ESSAQUI A., ZERTOUBI M., AZZI, M., BENAISSA M. 2014. *Study of antioxidant activity and anticorrosion action of the methanol extract of dwarf palm leaves (Chamaerops humilis* L.*) from Morocco [Étude de l'activité antioxydante et de l'action anti corrosion de l'extrait méthanolique des feuilles du palmier nain (Chamaerops humilis* L.*) du Maroc]*. J. Mater. Environ. Sci., 5 (2014): 887–898.

LABIAD M.H., HARHAR H., GHANIMI A., TABYAOUI M. 2017. *Phytochemical screening and antioxidant activity of Moroccan Thymus satureioïdes extracts*. J. Mater. Environ. Sci., 8(6): 2132–2139.

LISTER P. 2001. *Measurement of total phenolics and ABTS assay for antioxidant activity*. Crop Research Institute, Lincoln, New Zealand.

MOTTI R., ANTIGNANI V., IDOLO M. 2009. *Traditional plant use in the Phlegraean fields Regional Park (Campania, southern Italy)*. Hum Ecol., 37: 775–782. https://doi.org/10.1007/s10745-009-9254-1.

PASSALACQUA N.G., DE FINE G., GUARRERA P.M. 2006. *Contribution to the knowledge of the veterinary science and of the ethnobotany in Calabria region (Southern Italy)*. J. Ethnobiol. Ethnomed., 2(1): 1–14, https://dx.doi.org/10.1186%2F1746-4269-2-52.

QUÉZEL P., MÉDAIL F., LOISEL R., BARBERO M. 1999. *Biodiversity and conservation of forest species in the Mediterranean basin*. Unasylva., 50: 21–28. http://hdl.handle.net/10535/9505.

RENÉ M. 1957. *Encyclopédie biologique. Flore de l'Afrique du Nord*. Ed Paul Lechavalier. Encycl. biol. (Paris).

RAMMAL H., BOUAYED J., DESOR F., YOUNOS C., SOULIMANI R. 2009. *Notes ethnobotanique et phytopharmacologique de Hypericum perforatum L.* Phytothérapie., 7(3): 161, https://doi.org/10.1007/s10298-009-0385-8.

RHATTAS M., DOUIRA A., ZIDANE L. 2016. *Étude ethnobotanique des plantes médicinales dans le Parc National de Talassemtane (Rif occidental du Maroc)*. J. Appl. Biosci., 97: 9187–9211, http://dx.doi.org/10.4314/jab.v97i1.5.

RIAZ M., SHAHID M., JAMIL A., SAQIB M. 2017. *In vitro antioxidant potential of selected aphrodisiac medicinal plants*. J. Biol. Regul. Homeost. Agents, 31(2): 419–424, PMID: 28685547.

ROBY M.H.H., SARHAN M.A., SELIM K.A.-H., KHALEL K.I. 2013. *Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris* L.*), sage (Salvia officinalis L.), and marjoram (Origanum majorana* L.*) extracts*. Ind. Crops Prod., 43: 827–831, https://doi.org/10.1016/j.indcrop.2012.08.029.

SAVO V., LA ROCCA A., CANEVA G., RAPALLO F., CORNARA L. 2013. *Plants used in artisanal fishe-*

ries on the Western Mediterranean coasts of Italy. J. Ethnobiol. Ethnomedicine, 9(1): 9, http://doi.org/10.1186/1746-4269-9-9.

Sharififar F., Moshafi M.H., Mansouri S.H., Khodashenas M., Khoshnoodi M. 2007. *In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss*. Food Control., 18(7): 800– 805, https://doi.org/10.1016/j.foodcont.2006.04.002.

Sqalli H., El Ouarti A., Ennabili A., Ibnsouda S., Farah A., Haggoud A., Houari A., Iraqui M. 2007. *Evaluation de l'effet antimycobactérien de plantes du centre-nord du Maroc*. Bull Soc Pharm., 146: 271–288.

Sulaiman S.F., Sajak A.A.B., Ooi K.L., Seow E.M. 2011. *Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables*. J. Food Compos. Anal., 24(4–5): 506–515.

Sun B., Ricardo-Da-Dilva J.M., Dpranger I. 1998. *Critical factors of vanillin assay for catechins and proanthocyanidins*. J. Agric. Food Chem., 46(10): 4267–4274, https://doi.org/10.1021/jf980366j.

Tardío J., Pardo-de-Santayana M., Morales R. 2006. *Ethnobotanical review of wild edible plants in Spain*. J. Linn. Soc., 152(1): 27–71.

Topçu G., Ay M., Bilici A., Sarikürkcü C., Öztürk M., Ulubelen A. 2007. *A new flavone from antioxidant extracts of Pistacia terebinthus*. Food Chem., 103(3): 816–822, https://doi.org/10.1016/j.foodchem.29.028006.0.

Yam M.F., Ang L.F., Ameer O.Z., Salman I.M., Aziz H.A., Asmawi M.Z. 2009. *Anti-inflammatory and analgesic effects of Elephantopus tomentosus ethanolic extract*. J. Acupunct. Meridian. Stud., 2(4): 280–287.

Zielinski A.A.F., Haminiuk C.W.I., Nunes C.A., Schnitzle E., Van Rut S.M., Granato D. 2014. *Chemical composition, sensory properties, provenance, and bioactivity of fruit juices as assessed by chemometrics: a critical review and guideline*. Compr. Rev. Food Sci. Food Saf., 13(3): 300–316.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 271–282, Y. 2021

METALLIC BIOACCUMULATION IN *SESARMA HUZARDII* (DECAPODA: SESARMIDAE) FROM TWO ESTUARINE CREEKS UNDER DIFFERENT ANTHROPOGENIC INFLUENCES

Rasheed Olatunji Moruf

ORCID: 0000-0002-0459-0621
Department of Fisheries and Aquaculture
Bayero University, Kano, Nigeria

Key words: Abule-Agege Creek, Abule-Eledu Creek, crab, heavy metal, Lagos Lagoon.

Abstract

Heavy metals are of particular concern due to their toxicity and bio-accumulation ability in benthic organisms like aquatic crabs. This study analysed heavy metal concentrations in sediment and in species of mangrove crab, *Sesarma huzardii*, from Abule-Agege and Abule-Eledu Creeks by Atomic Absorption Spectrophotometry. The physico-chemical parameters (temperature, transparency, pH, conductivity, salinity and dissolved oxygen) of both creeks were not significantly different ($P > 0.05$). However, significantly higher concentrations of copper (5.08±0.08 mg kg^{-1}), iron (75.36±0.13 mg kg^{-1}) and zinc (14.5±0.11 mg kg^{-1}) were recorded in the sediment of Abule Agege Creek. All examined heavy metals were found in crab samples in varying and sometimes very low but measurable concentrations (except for nickel in Abule-Agege Creek). Bio-sediment accumulation factor (BSAF) of chromium (0.50) was the same in *S. huzardii* collected from both creeks. At the same time, the BSAFs of all other heavy metals were significantly different. The mangrove Crab, *S. huzardii* can be used for environmental monitoring largely because of its heavy metal bioaccumulation potential as a benthic organism.

Introduction

Mangrove ecosystems are found along tropical and subtropical coastlines all over the world, and they serve as nurseries for a range of marine vertebrate and invertebrate species (SANNI et al. 2020). The species of the mangrove crab (Sesarmidae) are amphibious and can be found around intertidal areas with moist/wet muddier regions of the mangrove. They live beneath drift and high tide mark in the estuaries and lagoons. Although these species does not really constitute a food item for the coastal

Address: Rasheed Olatunji Moruf, Bayero University, P.M.B. 53011, Gwarzo Road, Kano, Nigeria, phone: +2348022429983, e-mail: tunjimoruf@gmail.com

communities, they however play a major ecological role in the mangrove ecosystem where they help to clean up the mangrove areas by its feeding habits on the fallen leaves (Onadeko et al. 2015, Moruf and Ojetayo 2017). The species, *Sesarma huzardii* cohabiting with other mangrove crabs such as *Goniopsis pelli* and *Uca tangeri* have been found in swamps of the Lagos Lagoon, distributed up to the tidal limit in the lagoon (Moruf and Lawal-Are 2018, Lawal-Are et al. 2019a, Moruf 2020).

Over the years, several studies have emphasized the enormous threats posed to ecological receptors within the Lagos Lagoon and the adjacent creeks (Usese et al. 2018, Lawal-Are et al. 2021). About 2000 medium and large-scale industries in Lagos State discharge untreated effluents directly or indirectly into the Lagos Lagoon (Uaboi-Egbenni et al. 2010). The Ogun River carries wastes from the hinterland and discharges into the Lagoon. The effluents from Agbara Industrial Estate also drain into Lagos Lagoon through discharge into Ologe Lagoon, which is linked to Lagos Lagoon through Badagry creek (Uaboi-Egbenni et al. 2010). These contaminants have detrimental effects by accumulating in bottom sediments of the mangrove environment and altering its natural status (Bakshi et al. 2018, Moruf et al. 2021).

Aquatic organisms bioaccumulate contaminants such as heavy metals in minute amounts over time, and then get concentrated higher up the food chain (biomagnification). Accumulation of heavy metals begins when the organisms are faced with high concentrations in the surrounding medium; according to Moruf and Akinjogunla (2019), body levels of non-essential metals such as cadmium and lead were not found to be regulated by crustaceans. With respect to Lagos Lagoon, information exists on the occurrence of heavy metals in the muscle tissue and shells of gastropod molluscs (Moruf and Akinjogunla 2018), levels of oxidative stress markers in the mangrove oyster (Usese et al. 2019), and crab responses to environmental stressors (Lawal-Are et al. 2019b). However, comparative data are scarce on heavy metal accumulation in the tissues of mangrove crabs from different creeks adjacent to the Lagos Lagoon. Therefore, this study aimed to investigate and compare metal bioaccumulation in the visceral of the mangrove crab, *S. huzardii* from two estuarine creeks, Abule-Agege and Abule-Eledu, adjacent to the Lagos Lagoon in Lagos, Nigeria.

Materials and Methods

Study site

Two sites with different levels of anthropogenic influences were selected on the coast of Lagos: Abule-Agege and Abule-Eledu Creeks. They form part of the many sluggish tidal creeks that drain into the Lagos Lagoon. Five different study sites (600 m^2 each) located along each creek were surveyed (Figure 1). Each sample location was georeferenced with a Magellan Sport Track global positioning system (GPS) with accuracy of a metre. Abule–Agege Creek is located on Latitude 6°30′53″N and Longitude 3°24′44″E while Abule-Eledu Creek is located on Latitude 6°31′20″N and longitude 3°23′95″E. The creeks are shallow (≤ 1m) and meander through a mangrove swamp inundated at high tide and partially exposed at low tide (EMMANUEL and OGUNWENMO 2010, MORUF et al. 2018). The sources of pollution to the sampling stations include solid waste, household waste and refuse dumping.

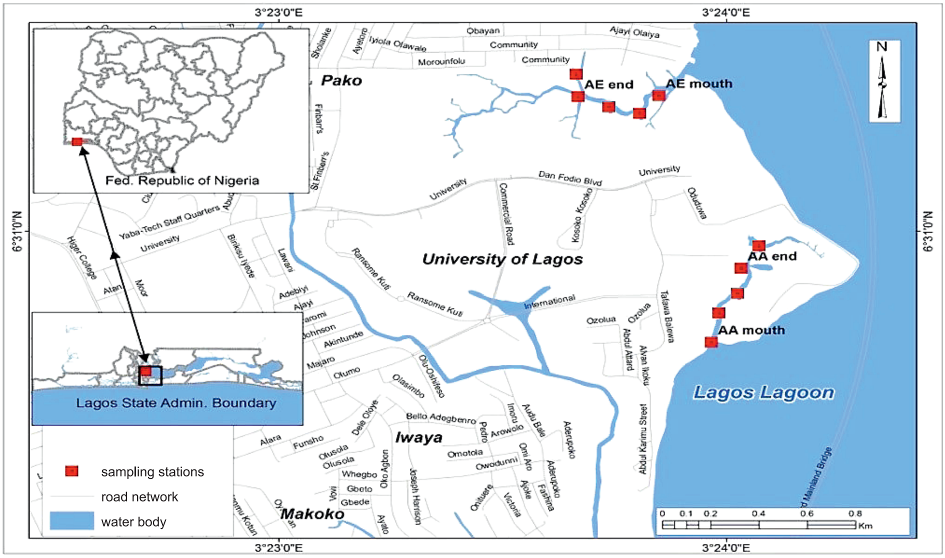


Fig. 1. Map showing the study area (red dots indicating the study site)

Collection of samples

A monthly collection of samples was carried out from January to June 2019. Water and surface sediment (at a depth of 2 cm) were collected using bottles with glass stoppers (250 ml reagent bottles and 1,500 ml polypropylene plastic containers) and 0.05 m^2 Eckman Grab, respectively. Five representative water and sediment samples from each of the five sampling sites from both creeks were taken. A total of 158 adult specimens of *S. huzardii* (80 from AA Creek and 78 from AE Creek) were hand-picked using protective rubber gloves. All samples were transported in a cooling box to the laboratory immediately after collection. The examined crab samples ranged in carapace length between 1.8 cm and 5.5 cm, while weight was between 4.8 g and 34.4 g.

Water quality analysis

Water samples were analyzed for physico-chemical parameters using the methods recommended by APHA (2005). The temperature was measured in-situ using a mercury-in-glass thermometer, and readings were taken to the nearest 0.1°C. In addition, salinity, pH, dissolved oxygen, and conductivity were measured using a refractometer (Model No: RHS-10), pH meter (Model: HI 2210), Lutron DO meter (Model: DO 5519), and conductivity meter (Model: EC 215), respectively.

Heavy metal analysis

Tiny stones were carefully removed from each bulk sediment sample and oven dried to constant weight at 105ºC. Each of the dried bulk samples was crushed separately with a clean pestle and mortar to homogenize it. Approximately 5 g of sediment was weighed into a crucible and heated to carefully burn the sample. A muffle furnace was used to heat the residue at 550ºC for complete oxidizing of carbon content (about 1 h). A few drops of Aqua-regia were used to dissolve the residues, followed by dilution with water. The resulting mixture was then filtered, rinsed thoroughly, and the filtrate was made up to the 100 ml mark in a standard (volumetric) flask. The resulting solution from the digestion was then analyzed with Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Analyst 200 using air-acetylene flame.

The muscle tissue of the crab samples was oven dried to constant weight at 105°C. The dried samples from each station were ground into a fine powder with a pestle and mortar, placed in bottles, and labeled. One gram of homogenized sample was digested with 8 mL HNO_3 and 2 mL H_2O_2 using a four-step digestion program in a microwave digestion system (MARSXpress, CEM, USA), performed according to the procedure of Turkmen and Ciminli (2007). After calibration, samples were analyzed for chromium, copper, iron, lead, nickel, manganese, and zinc by atomic absorption spectrophotometer.

Bio-sediment accumulation factor (BSAF), which is the ratio of the metal concentration in crab tissue to the metal concentrations in sediment (DEFOREST et al. 2007) was also calculated as:

$$\text{BSAF} = \frac{heavy\ metal\ concentration\ in\ crab}{heavy\ metal\ convcentration\ in\ sediment}$$

Data analysis

Normality of data was tested using the Kolmogorove-Smirnov Test ($\alpha = 0.05$). Homogeneity of variance was tested using Levine's test. Differences between mean concentrations in samples from the two different sampling locations were tested using T-test ($\alpha = 0.05$). Statistical analyses were performed with Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 12.0.1.

Results

Physico-chemical variables of two estuarine creeks

The data obtained for physico-chemical parameters at Abule-Agege and Abule-Eledu Creeks from January to June 2019 are represented in Table 1. There was slight variability in the mean parameter values obtained at the study zones. More so, all the investigated parameters were not statistically significant ($p > 0.05$) between both creeks. Abule-Agege Creek was higher in mean values of air temperature (29.90±0.01°C), water temperature (29.00±0.30°C), transparency (34.10±4.50 cm), conductivity (12470.68±112.10 µs cm^{-1}), salinity (8.60±7.10 ppt) and dissolved oxygen (2.90±0.01 mg L^{-1}). At Abule-Eledu Creek, the pH values (at 25°C) revealed that the water was slightly alkaline, with a range of 7.03–8.30. The mean value of dissolved oxygen was generally low, with a range varying between 3.60 mg L^{-1} and 4.20 mg L^{-1} across zones.

Table 1
Mean (±SE) and range (in bracket) of physico-chemical parameters of two estuarine creeks in Lagos, Nigeria

Parameter	Abule-Agege Creek	Abule-Eledu Creek	*P*-value (*P* < 0.05)*
Air temperature [°C]	29.90±0.01 (27.22–31.20)	29.12±0.09 (27.10–30.08)	0.30
Water temperature [°C]	29.00±0.30 (27.60–28.20)	28.40±0.01 (26.10–27.03)	0.24
Transparency [cm]	34.10±4.50 (28.98–39.80)	33.90±5.20 (27.90–36.50)	0.92
pH at 25°C	7.80±0.50 (7.10–8.50)	7.90±0.01 (7.03–8.30)	0.32
Conductivity [µS cm^{-1}]	12470.68±112.10 (2141.10–2400.20)	11087.52±101.80 (1234.20–0540.30)	0.32
Salinity [ppt, at 25°C]	8.60±7.10 (5.90–13.70)	7.60±5.30 (5.10–13.20)	0.34
Dissolved oxygen [mg L^{-1}]	4.0±0.01 (3.70–4.20)	3.90±0.05 (3.60–4.05)	0.25

Heavy metal concentration in sediment of two estuarine creeks

Table 2 shows the concentration of heavy metals (chromium, copper, iron, lead, nickel, manganese and zinc) in sediment samples collected from the intertidal zone of Abule-Agege and Abule-Eledu Creeks.

Table 2
Concentration of heavy metals (mean±SE) in sediment samples from two estuarine creeks in Nigeria

Heavy metals [mg kg^{-1}]	Abule-Agege Creek	Abule-Eledu Creek	*P*-value (*P* < 0.05)*
Chromium	0.04±0.01	0.06±0.03	0.18
Copper	5.08±0.08	1.41±0.05	0.04*
Iron	75.36±0.13	36.58±0.11	0.01*
Lead	0.03±0.01	0.01±0.01	0.09
Nickel	0.01±0.05	0.02±0.02	0.00*
Manganese	1.64±0.01	0.67±0.05	0.08
Zinc	14.5±0.11	7.8±0.15	0.03*

Higher concentrations [mg kg^{-1}] of copper (5.08±0.08), iron (75.36±0.13), lead (0.03±0.01), manganese (1.64±0.01) and zinc (14.5±0.11) were found at Abule-Agege Creek while higher mean values of chromium (0.06±0.03) and nickel (0.02±0.02) was observed at Abule-Eledu Creek. The significantly higher concentration of copper, iron, and zinc at Abule-Agege Creek implies that anthropogenic contribution to the heavy metal loads was clearly noticed in sediments.

Heavy metal concentration in visceral samples of *Sesarma huzarddii*

Table 3 shows the concentrations of heavy metals [mg kg^{-1}] in the visceral of the mangrove crab (*S. huzardii*) collected from Abule-Agege and Abule-Eledu Creeks. With the exception of chromium (0.02±0.05 mg kg^{-1}), non-significant ($p > 0.05$) higher mean values of heavy metals were found in *S. huzardii* collected from Abule-Agege Creek, for copper (3.27±0.08 mg kg^{-1}), lead (0.02±0.01 mg kg^{-1}), manganese (0.84±0.01 mg kg^{-1}) and zinc (5.06±0.18 mg kg^{-1}). Nickel was not detected in the crab samples from Abule-Agege Creek, but the mean value of 0.01±0.05 mg kg^{-1} was measured in the crab samples from Abule-Eledu Creek. However, a significantly higher concentration of iron (36.46±0.16 mg kg^{-1}) was detected in crab samples from Abule-Agege Creek.

Table 3

Concentration of heavy metals (mean±SE) in visceral samples of *Sesarma huzardii* from two estuarine creeks in Nigeria

Heavy metals [mg kg^{-1}]	Abule-Agege Creek	Abule-Eledu Creek	*P*-value ($P < 0.05$)*
Chromium	0.02±0.05	0.03±0.03	0.21
Copper	3.27±0.08	1.33±0.05	0.07
Iron	36.46±0.16	10.41±0.09	0.00*
Lead	0.02±0.01	0.01±0.01	0.12
Nickel	ND	0.01±0.05	0.00*
Manganese	0.84±0.01	0.5±0.01	0.07
Zinc	5.06±0.18	4.07±0.15	0.13

ND – not detected

Bio-sediment accumulation factor of heavy metals in *S. huzardii*

The bio-sediment accumulation factor (BSAF) of heavy metals in *S. huzardii* can be seen in Figure 2. Except for nickel (in Abule-Agege Creek), all examined heavy metals were observed to bio-accumulate in measurable concentrations in sampled crabs across the creeks. Confirming the clue from the standard deviation error bars (no overlapping), *t*-test was used to draw a conclusion. BSAF of chromium (0.50) was found to be the same in *S. huzardii* from both creeks, while the BSAFs of all other heavy metals were significantly different ($P < 0.05$). Except for BSAF of iron, all other BSAFs were higher in crabs collected from Abule-Eledu Creek.

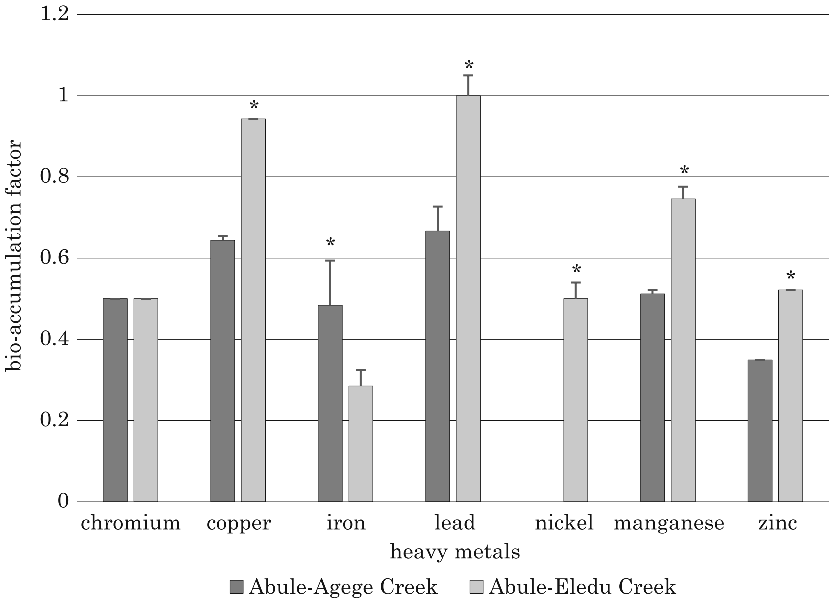


Fig. 2. Bio-sediment accumulation factor of *Sesarma huzardii* from two estuarine creeks

Discussion

The variations among the investigated physico-chemical parameters of both creeks were not statistically significant ($P > 0.05$). However, most of the investigated parameters were slightly higher in Abule-Agege Creek. The temperature range was within the tropical climate range of < 40°C for coastal waters as stated by FMENV (2001). The decrease or increase in water temperature depends mainly on the climatic conditions sampling times, sunshine hours and affected by specific characteristics of water environment such as turbidity, wind force, plant cover and humidity (AHMED et al. 2017, LAWAL-ARE et al. 2021). A similar observation was reported by ADEJUMOBI et al. (2019) for Makoko Creek in Lagos, Nigeria. Water temperature is easily influenced by turbidity, vegetation cover, run-off, inflows, and heat exchange with the air (OBOH and AGBALA 2017).

In this study, high transparency values confirm the known phenomenon that transparency and rainfall are inversely related to the region, as stated by LAWAL-ARE et al. (2019c). Furthermore, the creeks exhibited the usual alkaline properties and correspond with EMMANUEL and OGUNWENMO (2010), who reported pH values ranging between 7.30 and 9.20. This alkaline pH may be due to the buffering effects of the seawater. Conductivity and salinity have been previously reported as associated factors in the tidal creeks of Lagos Lagoon (ONYEMA et al. 2010). These parameters are similar in this study for both creeks. To a large extent, these variations in conductivity and salinity could be attributed to the effect of tidal seawater incursion and freshwater input from adjoining creeks and land as expected during the dry season (ONYEMA et al. 2010). According to LAWAL-ARE et al. (2010), salinity is an environmental barrier in the distribution of crabs. The lower values of dissolved oxygen recorded at Abule-Eledu Creek could be attributable to the consumption of the dissolved oxygen by aerobic microorganisms which biodegrade the organic wastes. Similar observations were reported by NKWOJI (2016) at Iddo, Ogudu and Agboiyi Creeks of Lagos Lagoon.

Heavy metals are of particular concern due to their toxicity and ability to be bio-accumulated in benthic organisms (MORUF and DUROJAIYE 2020). Sediment has been known to be the major depository of metals, holding more than 99 percent of the total amount of metal present in the aquatic system (ADERINOLA et al. 2009). The result from this present study revealed measurable concentrations of heavy metal in the sediment of Abule-Eledu Creek. At the same time, nickel was not detected in the sediment of Abule-Agege Creek. Higher concentrations of all the heavy metals (except chromium) were recorded in the sediment of Abule-Agege Creek than in the sediment of Abule-Eledu Creek, which in turn reflected on the bioaccumulation level in the mangrove crabs. Amongst all the metals analyzed, iron was observed to have the highest value. This was in agreement with the study of ONWUTEAKA et al. (2015), where the level of iron was the highest metal contamination in both water and sediment of the brackish water creek system of the Niger Delta. UABOI-EGBENNI et al. (2010) also reported iron with the highest heavy metal concentrations in Lagos Lagoon.

The present study examined heavy metals accumulated in the sampled crab, *S. huzardii,* to varying degrees. Lead > copper > manganese > chromium > iron > zinc > nickel were the heavy metal bio-accumulation patterns discovered in crabs from Abule-Agege Creek, in decreasing order. Heavy metal bioaccumulation patterns in crabs from Abule-Eledu Creek, on the other hand, followed a decreasing order of lead > copper > manganese > zinc > nickel/chromium > iron. This pattern is similar to that reported by Moruf and Akinjogunla (2019) on heavy metal accumulation in *Farfantepenaeus notialis* from two interconnecting brackish/freshwater lagoons in Lagos, Nigeria.

The heavy metals' bio-sediment accumulation factors (BSAF) showed that the crab visceral accumulated all the investigated heavy metals (except nickel in Abule-Agege Creek) in varying concentrations with lead as the highest, while iron was recorded as the lowest accumulated heavy metals in Abule-Eledu Creek. The BSAFs of the heavy metals (except chromium) were significantly different in crabs collected from both creeks. The heavy metal concentrations in the mangrove crab, *S. huzardii* may be attributed to the bio-magnification of metals in the biota. In contrast, the higher concentrations of metals in sediment may be attributed to metal-contaminated phytoplanktons that die and are deposited in the sediment.

Conclusion

The physico-chemical parameters estimated in this study did not significantly ($P > 0.05$) change across both Abule-Agege and Abule-Eledu Creeks, suggesting similar regional geological zone. It was found that the concentration of copper, iron and zinc were significantly ($P < 0.05$) higher in the sediment of Abule-Agege Creek than that of Abule-Eledu Creek. Except for nickel in Abule-Agege Creek, all examined heavy metals were observed to bio-accumulate in measurable concentrations in sampled crabs across the creeks. The mangrove crab, *Sesarma huzardii* can be used for environmental monitoring, largely because of its heavy metal bioaccumulation potential as a benthic organism.

Acknowledgement

The author is indebted to the Department of Marine Sciences, University of Lagos and the Head of Shellfish Unit, Prof. (Mrs) A.O. Lawal-Are for facilitating this research. Also, I wish to appreciate the editorial assistance rendered by Hajia Hawwau Moruf of the Department of Library and Information Science, Federal University Dutsin-Ma, Katsina State, Nigeria.

Accepted for print 6.10.2021

References

ADEJUMOBI I., NWANKWO D.I., ADEDIPE J. 2019. *Environmental characteristics, plankton diversity and seasonal variation at the Makoko Creek*. International Journal of Fisheries and Aquatic Studies, 7(3): 203–212.

ADERINOLA O.J., CLARKE E.O., OLARINMOYE O.M., KUSEMIJU V., ANATEKHAI M.A. 2009. *Heavy metals in surface water, sediments, fish and Perwinkles of Lagos Lagoon*. American-Eurasian Journal of Agricultural and Environmental Science, 5(5): 609–617.

AHMED M.H., EL-HAMED A., NADIA N.B. SHALBY N.I. 2017. *Impact of physico-chemical parameters on composition and diversity of zooplankton community in Nozha hydrodrome, Alexandria, Egypt*. Egyptian Journal of Aquatic Biology and Fisheries, 21(1): 49–62.

APHA (American Public Health Association). 2005. *Standard methods for the examination of water and wastewater*. 21^{st} ed. American Public Health Association. Washington DC.

BAKSHI M., GHOSH S., CHAKRABORTY D., HAZRA S., CHAUDHURI P. 2018. *Assessment of potentially toxic metal (PTM) pollution in mangrove habitats using biochemical markers. A case study on Avicennia officinalis* L. *in and around Sundarban, India*. Marine Pollution Bulletin, 133:157–172.

DEFOREST D.K., BRIX K.V., ADAMS W.J. 2007. *Assessing metal bioaccumulation in aquatic environments. The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration*. Aquatic Toxicology, 84(2): 236–246.

EMMANUEL B.E., OGUNWENMO C.A. 2010. *The macrobenthos and the fishes of a tropical estuarine creek in Lagos, South Western Nigeria*. Report and Opinion, 2(1): 6–13.

FMENV (Federal Ministry of Environment) (2001). *National guidelines and standards for water quality in Nigeria*. Technical Advisory Committee on Water Quality Criteria, Federal Ministry of Environment, Abuja, Nigeria.

LAWAL-ARE A.O., ONYEMA I.C., AKANDE T.R. 2010. *The water chemistry, crustacean zooplankton and some faunal species of a tropical tidal creeks in Lagos, Nigeria*. Journal of American Science, 6(1): 81–90.

LAWAL-ARE A.O., MORUF R.O., AKUBUEZE K.L., ADEWOLE O. 2019a. *Morphometric characteristics of two walking crabs, Cardiosoma armatum (Gecarcinidae) and Goniopsis pelii (Graspidae) from a tropical mangrove swamp in Lagos, Nigeria*. Nigerian Journal of Agriculture, Food and Environment, 15(1): 83–88.

LAWAL-ARE A.O., MORUF R.O., OLUSEYE-ARE S.O., ISOLA T.O. 2019b. *Antioxidant defense system alternations in four crab species as a bio-indicator of environmental contamination*. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 76(1): 73–80.

LAWAL-ARE A.O., MORUF R.O., AMOSU A.I., SADIQ S.O. 2019c. *Dynamics of crustacean larvae composition and abundance in mesohaline creeks of Lagos Lagoon, Nigeria*. Egyptian Academic Journal of Biological Sciences, B. Zoology, 11(2): 99–110.

LAWAL-ARE A.O., MORUF R.O., SOBARA U.J., SALAMI K.B. 2021. *Relationship between mercury concentration in water, bottom sediment and two mollusc species (Crassostrea gasar and Tympanotonus fuscatus) from a Lagos creek in Nigeria*. Journal of Bio-Science, 29(1): 143–151.

MORUF R.O. 2020. *Estimation of selected biological parameters of the Purple Mangrove Crab, Goniopsis pelii (Herklots, 1851) from a tropical mangrove swamp in Nigeria*. Ruhuna Journal of Science, 11(1): 13–22.

MORUF R.O., AKINJOGUNLA, V.F. 2018. *Photometric determination of macro-micro minerals in the West African mud creeper, Tympanotonus fuscatus var radula (Linnaeus, 1758)*. Journal of Experimental Research, 6(3): 35–40.

MORUF R.O., AKINJOGUNLA V.F. 2019. *Concentration of heavy metals in sediment of two interconnecting brackish/freshwater lagoons and the bioaccumulation in the crustacean, Farfantepenaeus notialis (Pérez-Farfante, 1967)*. Journal of Fisheries and Environment, 43 (3): 5 5–62.

Moruf R.O., Bolaji O.D., Lawal-Are A.O. 2018. *Biometrics, gut contents and sexual dimorphism of the West African Mud Creeper, Tympanotonus fuscatus var radula from the mangrove swamps of a coastal estuary in Nigeria.* Egyptian Journal of Aquatic Biology and Fisheries, 22(1): 87–96.

Moruf R.O., Durojaiye A.F. 2020. *Health risk appraisal of selected heavy metals in edible aquatic molluscs of Lagos, Nigeria.* Fudma J. Agric & Agric. Tech., 6(1): 42–48.

Moruf R.O, Durojaiye A.F., Okunade G.F. 2021. *Metal contamination and health risks in West African Mud Creeper (Tympanotonos fuscatus var radula) from Abule-Agele Creek, Nigeria.* Bulletin of Environmental Contamination and Toxicology, pp. 1–8.

Moruf R.O., Lawal-Are, A.O. 2018. *Haematobiochemical variations in estuarine crabs from a lagoon ecosystem.* Journal of Applied Sciences and Environmental Management, 22(12): 1899–1903.

Moruf R.O., Ojetayo T.A. 2017. *Biology of the West African Fiddler Crab, Uca tangeri (Decapoda: Ocypodidae) from a mangrove wetland in Lagos, Nigeria.* International Journal of Aquatic Biology, 5(4): 263–267.

Nkwoji J.A. 2016. *Impact of hypoxia on the community structure of benthic macroinvertebrates of Lagos Lagoon, Nigeria.* Journal of Applied Sciences and Environmental Management, 20(1): 121–130.

Oboh I.P., Agbala C.S. 2017. *Water quality assessment of the Siluko River, Southern Nigeria.* African Journal of Aquatic Science, 42(3): 279–286.

Onadeko A.B., Lawal-Are A.O., Igborgbor O.S. 2015. *Habitat diversity and species richness of brachyuran crabs off University of Lagos Lagoon coast, Akoka, Nigeria.* The Bioscientist, 3(1): 14–28.

Onwuteaka J.N., Edoghotu A.J., Friday U., Wokoma A.O.F., Bob-Manuel F.G. 2015. *Heavy metals contamination of the sessile bivalve, Crassostrea gasar (mangrove oyster) in Ai post oil spilled brackish water creek of the Niger Delta, Nigeria.* Annals of Biological Research, 6(11): 72–77.

Onyema I.C., Nkwoji J.A., Eruteya O.J. 2010. *The water chemistry and plankton dynamics of a tropical high energy erosion beach in Lagos.* Journal of American Science, 6(1): 111–122.

Sanni Z.A., Moruf R.O., Lawal-Are A.O. 2020. *Hemato-biochemical profiling of a burrowing crab exposed to polystyrene microplastic contaminant.* FUDMA Journal of Sciences, 4(2): 380–385.

Turkmen M., Ciminli C. 2007. *Determination of metals in fish and mussel species by inductively coupled plasma-atomic emission spectrometry.* Food Chemistry, 103: 670–675.

Uaboi-Egbenni P.O., Okolie P.N., Martins O., Teniola O. 2010. *Studies on the occurrence and distribution of heavy metals in sediments in Lagos Lagoon and their effects on benthic microbial population.* African Journal of Environmental Science and Technology, 4(6): 343–351.

Usese A.I., Lawal-Are A.O., Moruf R.O., Chukwu L.O. 2018. *Biomarker responses to environmental stressors in the hairy mangrove crab, Sesarma huzardii (Graspidae) from a tropical lagoon mudflat in Nigeria.* Alexandria Journal of Veterinary Sciences, 57(1): 4–10.

Usese A.I., Elike M.I., Moruf R.O., Chukwu L.O. 2019. *Levels of oxidative stress markers in the mangrove oyster, Crassostrea gasar from a coastal ecosystem in Southwest Nigeria.* Journal of Research in Forestry, Wildlife & Environment 11(1): 32–38.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 283–298, Y. 2021

MORPHOLOGICAL CHARACTERISTICS OF THE CULTURE *CLATHRUS ARCHERI* (PHALLACEAE, BASIDIOMYCOTA)

***Oksana Mykchaylova*[1], *Margarita Lomberg*[2], *Sofiia Tsapko*[3], *Viktoriia Krasinko*[4]**

[1] ORCID: 0000-0001-9212-5094
[2] ORCID: 0000-0001-7036-7850
[3] ORCID: 0000-0003-0062-5177
[4] ORCID: 0000-0003-3653-8673
[1,2] Department of Mycology, M.G. Kholodny Institute of Botany
National Academy of Sciences of Ukraine, Kyiv, Ukraine
[3,4] Department of Biotechnology and Microbiology
National University of Food Technologies, Kyiv, Ukraine

Key words: gasteroid mushroom, pure culture, micromorphological characteristics, growth rate, temperature, *IBK*, *ex situ*, *in vitro*.

Abstract

This article touches upon the morphological and growth characteristics of the gasteroid mushroom *Clathrus archeri* from the *IBK* Mushroom Culture Collection (Ukraine). For fungus identification *in vitro*, the micromorphological properties of vegetative mycelium have been specified by light and scanning electron microscopy. The presence and structure of crystals, vesicular cells, septum swelling, and mycelial cords have been described. Experimental data on fungi growth rate were obtained on five agar nutrient media at different temperatures ranging from 4ºC to 28ºC. The temperature of 26ºC has been determined as optimal for mycelium incubation, and 39ºC – as critical for culture viability. The morphological characteristics of the fungi on various agar media have been reported. According to the radial growth rate within 0.1–2.9 mm/day, the investigated species belongs to the slow-growing fungi. Nutrient media such as wort agar and compost agar have appeared to be the most favorable for the vegetative growth of *C. archeri* mycelium.

Introduction

The studies on various aspects of biology and systematics of macromycetes traditionally involve the macroscopic and microscopic characteristics of fruit bodies for species identification. At the same in modern *in vitro* experimental studies; macromycetes at the vegetative stage of develop-

Address: Margarita Lomberg, M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereshchenkivska Str., 2, Kyiv 01004, Ukraine, e-mail: margarita@lomberg.kiev.ua

ment. Unfortunately, gasteroid saprotrophic macromycetes are studied insufficiently *in vitro*. It is due to the difficulties associated with pure culture isolation and deposition. The crucial criteria used for taxon characterization at the vegetative stage of development and culture identification remain ambiguous. One such inadequately investigated species at the vegetative stage of development is *Clathrus archeri* (Berk.) Dring (1980), (synonyms: *Anthurus archeri* (Berk.) E. Fisch., *Anthurus sepioides* McAlpine, *Aserophallus archeri* (Berk.) Kuntze, *Lysurus* archeri Berk, *Pseudocolus archeri* (Berk.) Lloyd, *Schizmaturus archeri* (Berk.) Locq.). Due to the unusual appearance of the fruit bodies, *Clathrus archeri* has several common names, such as "devil's fingers", "octopus stinkhorn", and "squidward mushroom". According to the Dictionary of Fungi, the *Clathrus* genus (Phallaceae, Phallales) comprises 16 species (SPECIES FUNGORUM 2021, *Catalogue of life* 2021) mainly found in subtropical and tropical regions. *C. archeri* undergoes two phases in the sporomic stage: a myco-egg phase and a receptacle phase (PIETRAS et al. 2016). This species is a saprotrophic gasteroid fungus belonging to ephemeral macromycetes with primary subtropical and tropical distribution. The fungi occur mainly in the southern hemisphere regions – in New Zealand, Australia, Tasmania, South America, Southern, and Eastern Africa, Mauritius, St Elena Islands (DRING 1980). The first findings of this species are dated back to 1914 in Europe on the territory of France. It is believed that the mycelium and spores of *C. archeri* may have been transferred from Australia and New Zealand to Europe with military fodder or wool for the textile industry (DRING 1980, ZYKOVA 2007). The following findings of the fungi were described on the territory of northeastern France in the 1920s–1930s (PARENT et al. 2000). Over the next few decades, *C. archeri* began spreading rapidly throughout Europe (from Spain to Scandinavia). The mechanism of that fast spread across Europe is not fully understood. Nevertheless, some scientists report on the specific odor of mature fruit bodies of fungus that attracts insects that spread the spores over a considerable distance (KRIEGLSTEINER 1992, JOHNSON and JÜRGENS 2010). In Ukraine, *C. archeri* was first registered in August 1977 near Onokovtsi village located in Uzhorod district of Zakarpattia region (HELUTA and ZYKOVA 2018). After that, there were no reports on new findings of this fungus in Ukraine. Given the low frequency of *C. archeri* occurrence, the third edition of the Red Data Book of Ukraine listed it as an endangered species with a disjunctive range (DUDKA 2009). In recent years, however, researchers have begun to record the mass formation of *C. archeri* fruit bodies in the anthropogenically modified Carpathian plant associations and adjacent areas. There are detailed literature reviews on this species spreading in Europe and Ukraine presented in the publications by Ukrainian mycologists (ZYKOVA 2007, HELUTA and ZYKOVA 2018). The vast majority of the published works are devoted to the systematics, ecology, distribution, and conservation status of this species in nature (ARORA et al. 1982, STENGL-REJTHAR and WOJEWODA 1985, HOSAKA et al. 2006, ZYKOVA 2007, DESPREZ-LOUSTAU 2009, BÎRSAN et al. 2014, STEBEL 2015, PIETRAS et al. 2016, HELUTA and ZYKOVA 2018, MATUS et al. 2018). However, the biological features of this fungus *in vitro* are almost unknown. According to the World Federation of Cultural Collections (WFCC), five strains of this species are maintained in the following official collections: Filamentous fungi and Yeast Collection in the Netherlands, Belgian Coordinated Collection of Microorganism (BCCM), Culture Collection of Basidiomycetes (CCBAS) of Institute of Microbiology in the Czech Republic, Culture Collection of Basidiomycetes of the Komarov Botanical Institute (LE BIN), All-Russian Collection of Microorganisms (WKM) both in Russian Federation, International Collection of Microorganisms from Plants (ICMP) in New Zealand. Dikaryotic strain *C. archeri* 2405, isolated from mycological material, gathered on the territory of Ukraine, is deposited in the *IBK* mushroom culture collection of M.G. Kholodny Institute of Botany, NAS of Ukraine (LOMBERG et al. 2015, BISKO et al. 2018). Given the limited knowledge of the cultural and morphological characteristics of *C. archeri*, our work aimed to determine the morphological and growth properties of the strain *C. archeri* 2405 on solid growth media of different compositions and to find out the optimal conditions for its deposition in the culture collection.

Materials and Methods

Pure culture isolation

The object of the study was *C. archeri* 2405 from the Collection of pileate fungi (*IBK*) (BISKO et al. 2021). Mycological material for *C. archeri* pure culture isolation was gathered during the expedition to Hutsulshchyna National Nature Park (15th October 2015). Young fungal fruits of *C. archeri* (in myco-egg phase) were collected in the Ivano-Frankivsk region, in the Shtefantsi mountain area located in Babyn village, Kosiv district, Hutsulshchyna National Nature Park (meadows, grazing). 48°16'02.4" N, 25°02'38.3" E.

Mycelial culture of *C. archeri* was obtained from the young fruit body of the fungi (Figure 1). In this development stage (myco-egg phase) fungal fruits have pear- or egg-like forms, are surrounded by a slime layer, and are covered with white dense peridium. The inoculum (pieces of gleba) was placed in the plates with wort agar (WA) media using sterile lancet. The inoculated plates were incubated at 25°C in a thermostat. Nutrient media were supplemented with penicillin (200 IU/ml) for bacterial growth inhibition.

Fig. 1. *Clathrus archeri* – the source of the strain *IBK* 2405
Source: photo by M.O. Zykova

The plates were incubated in the thermostat until the appearance of the colonies with a well-developed mycelium. The pure culture of fungi was maintained in the test tubes with WA media for culture deposition and follow-up studies. The absence of foreign microflora, morphological properties of colonies and vegetative mycelium were controlled visually and using a microscope.

Cultural and morphological investigations

For further characterization, cultures were inoculated onto different agar media with a pH adjusted to 6.0 in Petri dishes (diameter 90 mm, 20 mL per dish): malt extract agar (MEA, Merck, Germany); potato dextrose agar (PDA, DIFCO, USA); wort agar (8°Balling) (WA), wort agar supplemented with wheat straw (1%) (WAS), and glucose-peptone-yeast-agar (GPYA) consisted of (g/l): glucose – 25.0; peptone – 5.0; yeast extract – 3.0;

KH_2PO_4 –1.0; K_2HPO_4 – 1.0; $MgSO_4$ – 0.25; agar-agar – 20.0. Compost agar (CA) was prepared according to the method described by PRYDIUK and LOMBERG (2021). For CA, GPYA, WA, and WAS media preparation we gently mixed 1 L liquid and 20 g agar. All media were sterilized by autoclaving at 121°C for 30 min.

Surface cultivation was conducted at temperatures of 4±0.1, 18±0.1, 22±0.1, 26±0.1, and 28±0.1°C. Culture viability was checked by incubation on WA medium at 30–40°C in increments of 1±0.1°C. The presence or absence of mycelium growth was checked after ten days of incubation. The survival or viability loss of culture mycelium was examined by further cultivation at 26±0.1°C.

Growth rate and morphology study

The inoculum was prepared by cultivating *C. archeri IBK* 2405 mycelium on WA in darkness at 26°C for 14 days. 5 mm agar plugs were cut out from the actively growing part of a colony using a cork borer and placed into a center of the Petri dishes with a fresh medium (three cultures per isolate). The growth parameters of mycelia were analyzed weekly for up to a month according to a previously described method (LOMBERG and SOLOMKO 2012). The average radial growth rate (G_R) of mycelial colonies was determined by the formula:

$$G_R \text{ (mm/day)} = (R_2 - R_1)/(T_2 - T_1),$$

where R_2 and R_1 were the radii of the colonies (in mm) on the day of the beginning of growth (T_1) and the day of the last measurement of growth (T_2).

Microscopic investigations

Micro- and marcomorphological characteristics of the mycelium were examined on the 4-weeks old colonies by means of Stalpers scales (STALPERS 1978). Macromorphological features included a description of colony type, color and density, mycelium odor, the color of the reversum and the presence or absence of concentric circles. Micromorphological properties involved a description of hyphae system features, and occurrence of clamps and anamorphs on the mycelium. Microstructures of the vegetative mycelium of *C. archeri* were observed using optical microscope MBI-15 (Russia) and scanning electron microscope JSM-6060 LA 4 nm (JEOL, Japan) according to the modified method by Quattlebaum E. and Carner G. (BUCHALO et al. 2009).

Statistical analysis

The statistical analysis of obtained results was performed by standard methods using Student t-criteria by means of Microsoft Excel software and StatSoft Statistica 6.0. The values of standard deviations (SD), coefficients of variation, confidence intervals were calculated. Experimental data were expressed from quintuple measurements as mean ± SD. The values at $P < 0.05$ were considered significant.

Results and Discussion

Cultural and morphological examination of *C. archeri IBK* 2405 on standard agar growth media of different compositions – WA, MEA, PDA, GPYA, CA showed the variability of the morphological features of mycelial colonies depending on the compounds of the nutrient medium. On WA, PDA, CA media the strain formed dense colonies with numerous radial silky cords, white-colored at the early stage of growth, and pink shaded over time, with the zones of high tangled hyphae. The colony rim was uneven and raised over the substrate, the reverse matched the color of the substrate (Figure 2*a*). On GPYA medium the culture formed dense, velvety colonies with a noticeable concentric zonal sequence and a few short aerial hyphae along the rim of the colony. The mycelium acquired a pink-cream color with age, the rim was uneven and raised, the reversum matched the color of the substrate (Figure 2b).

The least cultivatable media for *C. archeri IBK* 2405 was MEA with very slow growth observed. Even on the 30th day of cultivation, the diameter of the colony did not exceed 20 mm. The culture formed white, dense, woolly colonies that acquired pink color with age, and had drops of exudate appeared. The colony rim was uneven, the reversum matched the substrate color (Figure 2c).

Fig. 2. Mycelial colonies of *Clathrus archeri IBK* 2405 on solid media: *a* – WA; *b* – GPYA; *c* – MEA at incubation temperature 26±0.1°C (30th day of cultivation)

We have examined the effect of various incubation temperatures on the radial growth rate and the morphology of the colonies of *C. archeri* 2405. The optimum temperature for growth was 26°C (Table 1). Based on the results of a study of *C. archeri* 2405 growth on agar growth media of different compositions, *C. archeri* 2405 can be attributed to the group of slowly growing fungi evidenced by its radial growth rate (Table 1).

Table 1

Radial growth rate (G_R, mm/day) of vegetative mycelium of *Clathrus archeri IBK* 2405 on agar nutrient media at different incubation temperature

Incubation temperature [°C]	G_R, mm/day on different agar nutrient media				
	WA	MEA	GPYA	PDA	CA
18±0.1	2.2±0.1	0.1±0.2	1.1±0.2	0.5±0.2	2.4±0.2
22±0.1	2.4±0.1	0.3±0.1	1.3±0.1	0.8±0.1	2.5±0.2
26±0.1	2.6±0.1	0.4±0.2	1.8±0.2	1.1±0.2	2.9±0.1
28±0.1	2.5±0.2	0.1±0.2	1.4±0.1	1.0±0.1	2.6±0.2

The effects of critical temperatures on the viability of *C. archeri* 2405 were investigated. For culture viability examination, the strain was incubated on CA medium at 30–40°C in increments of 1°C. After the third day of incubation, the presence or absence of mycelium growth was taken into account. The survival or viability loss of the plant mycelium was tested by the following incubation at 26±0.1°C. The critical temperature for *C. archeri* 2405 strain was 39±0.1°C.

Using SEM, we have obtained new data on the micromorphology of *C. archeri in vitro*. Vegetative mycelium consists mainly of thin-walled, moderately branched, regularly separated, unvarnished generative hyphae with a diameter of (2.94)3.81–4.63(5.17) µm (Figures 3–5).

Based on literature sources, the red and orange colors of fungal mature fruit bodies are imparted by the presence of carotenes, for the most part, lycopene, and *β*-carotene. While *β*-carotene is peculiar to Phallaceae species *Mutinus caninus*, *M. ravenelii*, and *M. elegans*, lycopene is present in the closely related fungus *C. archeri* (Fiasson and Petersen 1973).

According to M. Nobles (Nobles 1971), the strains of the same species may vary in the texture and color of mycelial colonies. More stable taxonomic features are micromorphological properties of the hyphae system, growth rate, optimal growth temperature. As revealed by literature data, both the composition of the growth media and the incubation temperature has a strong impact on the culture growth rate (Lomberg and Solomko 2012). Information about the growth characteristics of *C. archeri* on agar media is available only in the article by Pasailiuk et al. (2018). Fungi

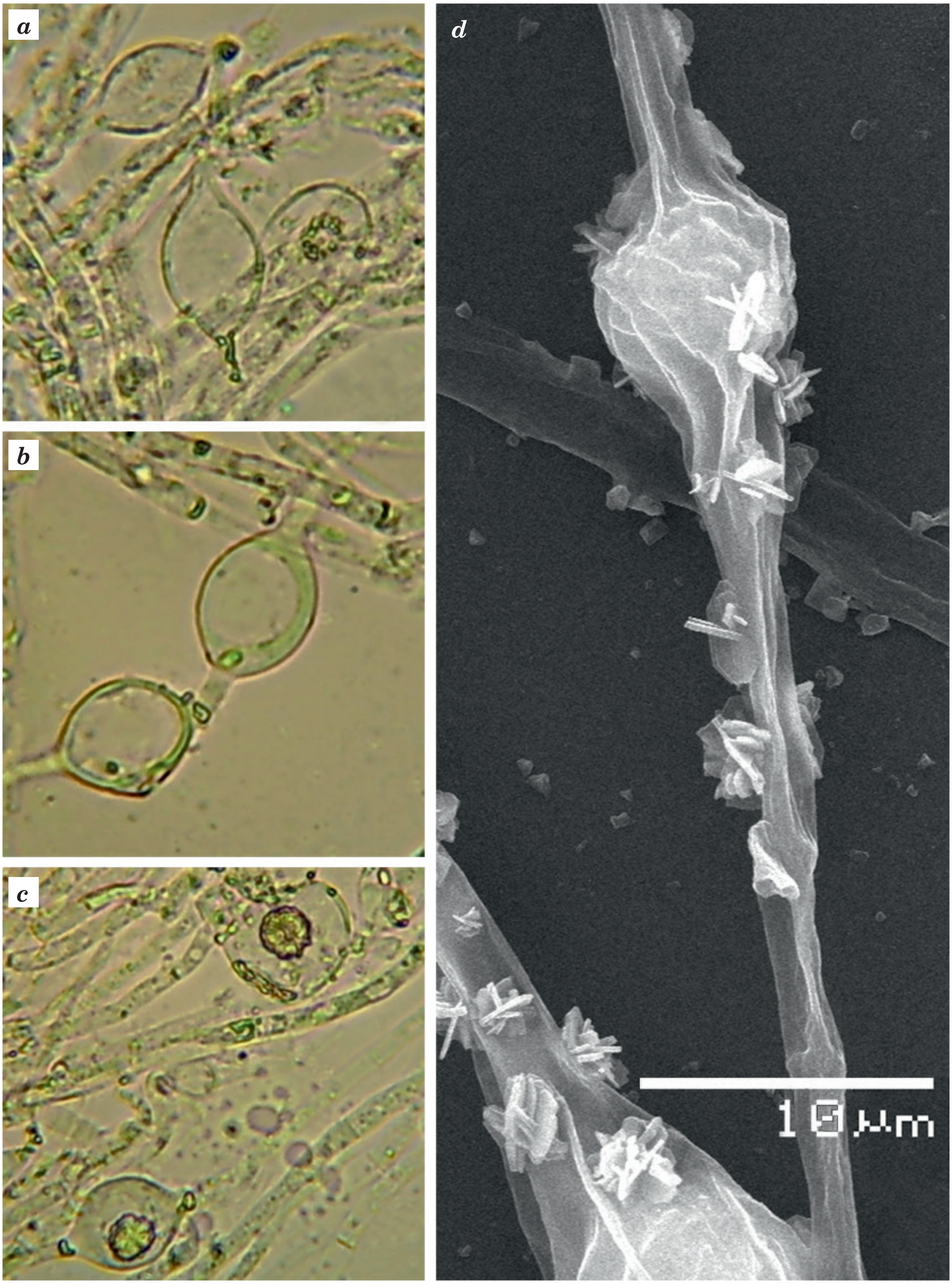


Fig. 3. *Clathrus archeri* IBK 2405: (*a*–*c*) vesicular cells (× 40); *c* – cells with oil droplets inside (× 40); *d* – crystals on hyphae; hyphae with irregular swelling in septum (SEM × 2200)

mycelium develops under natural and artificial conditions exclusively within a certain temperature range. The identification of this substantial environmental factor is needed to provide the optimal condition for the cultivation and deposition of fungi cultures. The effect of temperature on the growth and development of *C. archeri* has been studied fragmentarily. The majority of the published material on this topic is devoted to the observations of the appearance and development of *C. archeri* fruit bodies in the wild.

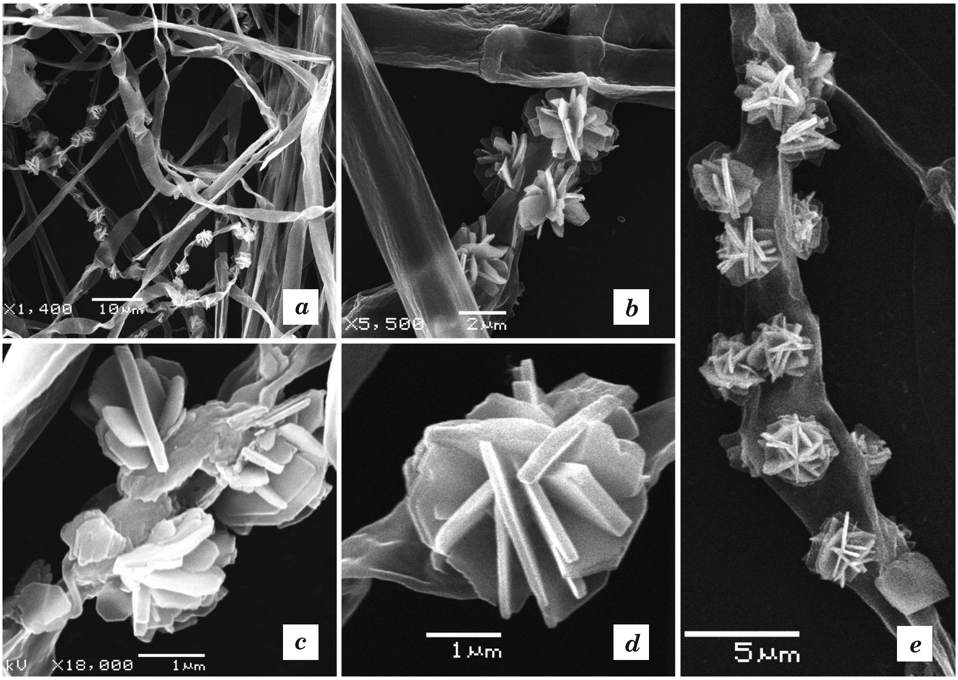


Fig. 4. *Clathrus archeri IBK* 2405: *a–e* – crystals on hyphae; *a*, *b* – septa without clamp cells

This species characterizes by the formation of rounded large vesicular cell of width (7.32)12.96–14.95(16.48) and length (19.26)25.49–27.74(30.92) µm. We observed large vacuoles and oil droplets in the middle of these cells (Figure 3). The presence of such cells was previously reported for *Phallus impudicus L.*, *Ph. hadrianii*, *Lycoperdon perlatum* Pers. (BUCHALO et al. 2009, DYAKOV et al. 2010, BUKO et al. 2019). One of the most requisite taxonomic features when identifying basidium macromycetes in the culture is the presence of clamps, formed on the hyphae of the dikaryotic mycelium of many members of this group of fungi (STALPERS 1978, BUCHALO et al. 2009). Different species feature specific differences in the position of the clamps on the hyphae, their location frequency, shape, size, etc. (Buchalo et al. 2009). However, in our study, we haven't observed any clamps formation in *C. archeri IBK* 2405. There was mainly clampless vegetative mycelium (Fig. 5). According to the reports, clamps are not also present in species of the genus *Armillaria, Lycoperdon perlatum Pers., L. pyriforme* Schaeff.

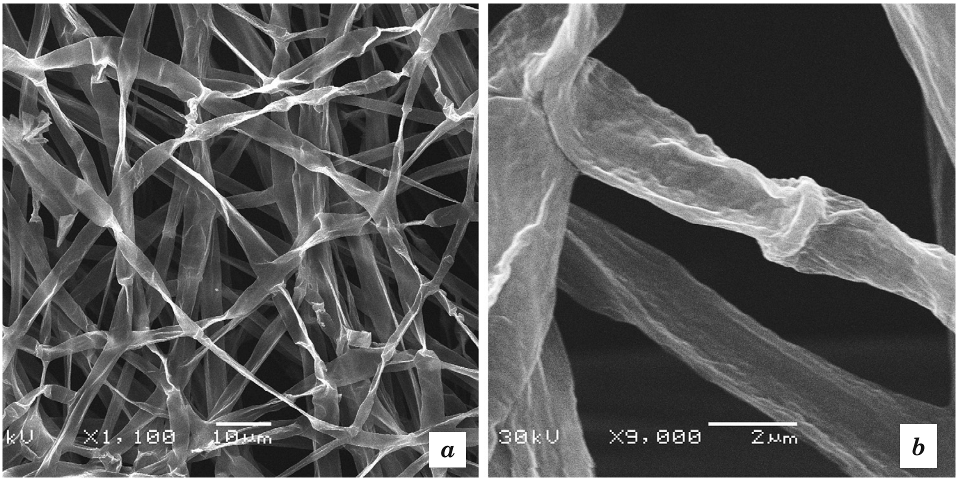


Fig. 5. Micromorphological structures in *Clathrus archeri IBK* 2405: *a* – clampless mycelia; *b* – anastomose and a septum inside the collapsed hypha

The morphological markings of secretory activity such as secretory hyphae and crystal deposition were observed in *C. archeri IBK* 2405 culture on the 20th day of cultivation. Numerous plate-like calcium oxalate crystals formed on the surface of hyphae, collected in bundles and resembled a flower (Figures 3, 4). *In vitro* crystal formation is described for fungi of different environmental groups (Arnott 1995, Arocena et al. 2001, Buchalo et al. 2009, Tuason and Arocena 2009, Badalyan and Borhani 2019). The density of the crystals on the surface of hyphae may vary. They grow inside the cell and exit it perpendicularly or at an angle to its surface. As a rule, the formed crystals remain on the surface of the cell wall and are rarely removed from it. As a result of hyphae lysis, crystals can occur in the environment. Some researchers suggest that the inlay of calcium oxalate in hyphae provides a hydrophobic protective layer on the surface of the fungus hyphae. It reduces the propensity of the hyphae when exposed to various microorganisms (Snetselaar and Whithney 1990, Arnott 1995). In a natural environment, the ability of fungi to form calcium oxalate crystals on the surface of the hyphae and secrete them into the substrate affects the biochemical processes in soils (Gadd et al. 2014). Mycelium acts as a place for calcium pooling, thereby changing the pH of the soil and the availability of phosphorus for plants (Cromack et al. 1979, Tuason and Arocena 2009). Based on the experimental data, it was concluded that oxalic acid dominates among other organic acids secreted by fungi in natural biogeocenoses. Oxalic acid performs different functions. Its geochemical value consists of metal cations binding and secondary mineral formation, as well as the dissolution of certain minerals and the conversion of some elements into forms accessible to fungi and plants. The acid can inhibit the growth of other microorganisms in the communities and can be a factor in allelopathy. The secretion of oxalic acid (the strongest organic acid) causes a rapid acidification of the external environment that may activate acidic hydrolases in the cell walls of plants, contributing to their destruction and penetration of fungi into plant tissues (Franceschi and Loewus 1995, Tuason and Arocena 2009). In addition, oxalic acid secreted by mycorrhizal fungi contributes to the establishment of a trophic relationship between plant roots and fungi hyphae.

Culture collections should ensure the security of intact biomaterial storage, relevant characterization, and information support of samples in modern databases. That is why the priority task of the macromycetes culture collections is the preservation of fungi strains *in vitro*, namely to support their purity, genetic stability, viability, and biological activity (Pearce et al. 2020). In world culture collections, various nutrient media are used for maintaining macromycetes in an active physiological state. According to reports, *C. archeri* strains are stored in the collections on standard nutrient media, such as WA (WKM, LE BIN), MEA (CCBAS), PDA, and Norkrans modified media (BCCM). When selecting the composition of the nutrient medium, we took into account the ecological suitability of *C. archeri* to natural substrates. In nature, the mass fruiting of *C. archeri* is associated with disturbed ecosystems that have noticeable anthropogenic impacts (tree felling, roads, grazing of livestock, landfills, etc.), are rich in organics – wood chips, sawdust, branches, leaves, dry grass, dead wood, and presumably have a high degree of substrate nitrification (Heluta and Zykova 2018). For this reason, we propose to use simultaneously three nutrient media for the storage of *C. archeri* strains: wart agar (WA), compost agar (CA), wart agar supplemented with wheat straw (1%) (WAS). CA and WAS have been successfully used in *IBK* for the storage and cultivation of *Agaricus bisporus* (JE Lange) Imbach, *Lepista nuda* (Bull.) Cooke, *Macrolepiota procera* (Scop.) Singer, *Phallus impudicus* L., *Lycoperdon perlatum* which like *C. archeri* belong to the environmental group of saprotrophic macromycetes.

In recent times, there were a few reports on the possibility of repeated reproduction of *Anthurus archeri* in the territory of the Hutsulshchyna

National Nature Park by applying the *re-situ* methodology (Pasailiuk et al. 2018). As a result of renaturalization actions, started in 2012, the annual fruiting of the fungus was obtained at the three mycological-reproductive sites. However, given that *C. archeri* is a species belonging to the alien mycobiota that aggressively exploits natural and man-made phytocenosis, its active spread constitutes a threat to natural phytocenosis. This species intensively inhabits azotized, affected areas: landfills, fellings, where its density reaches more than 10 copies per 100 m^2. The species occurs near settlements, avoiding natural cenosis, which indicates the signs of its adventisation. With this in mind, any introduction of these macromycetes into the ecosystems of Ukraine is wrong. From the standpoint of Ukrainian mycologists (Heluta and Zykova 2018), it is necessary to give up the idea of enriching the biodiversity of botanical gardens, dendrological parks, and other objects of the natural preservation fund with the macromycetes, including invasive ones. It is due to the fact that such contamination of mycobiota in Ukraine may become hazardous, resulting in unexpectedly severe and uncontrollable consequences. Considering the significant occurrence frequencyof this species in natural plant formations, *C. archeri* should be excluded from the Red Data Book of Ukraine as an alien and invasive species (Heluta and Zykova 2018).

Conclusions

The paper addresses the data on the growth and the morphology of *C. archeri IBK* 2405 culture on agar nutrient media of various compositions at different incubation temperatures. According to the growth rate, the strain belongs to the group of slow-growing fungi. The critical temperature for the viability of *C. archeri IBK* 2405 mycelium is 39±0.1°C.

The key micromorphological features of *C. archeri IBK* 2405 vegetative mycelium have been examined *in vitro* by means of scanning electron microscopy (SEM). This species is distinguished by a formation of roundish vesicular cells with large vacuoles and droplets of oil inside, along with numerous crystals.

The defined micro- and macromorphological properties of mycelium on the specific media and culture growth rate can be regarded as additional taxonomical characteristics of *C. archeri* at the vegetative stage of development. With ecological and biological peculiarities of *C. archeri* in mind, we have selected the composition of agar nutrient media for strain cultivation and preservation in the proper physiological state *in vitro*.

The specified biological characteristics are essential for establishing reliable means of pure culture maintenance in an artificial environment. It will contribute not only to the protection and genetic conservation of the fungi but their practical application as well.

Acknowledgments

The authors would like to acknowledge the staff of the Center for Collective Use of Electron Microscopes of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine for providing the SEM investigation and M.O. Zykova, PhD for providing photo of *C. archeri* carpophore in nature.

Accepted for print 27.09.2021

References

Arnott H.J. 1995. *Calcium oxalate in fungi*. In: *Calcium oxalate in biological systems*. Ed. S.R. Khan, CRC Press, Boca Raton, FL, USA, pp. 73–111.

Arocena J.M., Glowa K.R., Massicotte H.B. 2001. *Calcium-rich hypha encrustations on Piloderma*. Mycorrhiza, 10(5): 209–215.

Arora D., Burk W.R. 1982. *Clathrus archeri, a stinkhorn new to North America*. Mycologia, 74(3): 501–504.

Badalyan S.M., Borhani A. 2019. *Morphological and growth characteristics of mycelial collections of medicinal xylotrophic mushrooms (Agaricomycetes) distributed in forests of Northern Iran*. Chemistry and Biology, 53(2): 97–106.

Bîrsan C., Cojocariu A., Cenuşâ E. 2014. *Distribution and ecology of Clathrus archeri in Romania*. Notulae Scientia Biologicae, 6(3): 288–291.

Bisko N.A., Lomberg M.L., Mykchaylova O.B., Mytropolska N.Yu. 2021. *IBK Mushroom Culture Collection. Version 1.2. The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany*. Occurrence dataset https://doi.org/10.15468/dzdsqu accessed via GBIF.org on 2021-04-15.

Bisko N.A., Sukhomlyn M.M., Mykchaylova O.B., Lomberg M.L., Tsvyd N.V., Petrichuk Yu.V., Al-Maali G.A., Mytropolska N.Yu. 2018. *Ex situ conservation of rare and endangered species in mushroom culture collections of Ukraine*. Ukrainian Botanical Journal, 75(4): 338–347.

Buchalo A.S., Mykchaylova O.B., Lomberg M.L., Wasser S.P. 2009. *Microstructures of vegetative mycelium of macromycetes in pure cultures*. Kyiv, M.G. Kholodny Institute of Botany, p. 224.

Buko V., Bakunovich A., Astrowski A., Moroz V., Puchkova T., Kastsianevich A., Tomulewicz M. 2019. *Polysaccharides of mushroom phallus impudicus mycelium: immunomodulating and wound healing properties*. Modern Food Science and Technology, 35(9): 30–37.

Catalogue of life. 2021 (on-line), https://www.catalogueoflife.org/data/search? TAXON_ID=3QL5&rank=species&status=accepted&status=provisionally%20accepted, access: 15.05.2021.

Cromack Jr. K., Solkins P., Grausten W.C., Speidel K., Todd A.W., Spycher G., Li C.Y., Todd R.L. 1979. *Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus Hysterangium crassum*. Soil Biol. Biochem., 11: 463–468.

Desprez-Loustau M.L. 2009. *Alien fungi of Europe, Handbook of alien species in Europe*. Springer, Dordrecht, pp. 15–28.

Dring D.M. 1980. *Contributions towards a rational arrangement of the Clathraceae*. Kew Bull., 35(1): 1–96.

Dyakov M.Yu., Kamzolkina O.V., Shtaer O.V., Bisko N.A., Poyedinok N.L., Michailova O.B., Efremenkova O.V. 2010. *Morphological properties of some Basidiomycetes natural strains and biological analysis of their antimicrobial activity during submerged cultivation*. Mikologiya i Fitopatologiya, 44(3): 225–239.

Dudka I.O. 2009. *Anthurus archeri*. [In:] *Chervona knyha Ukrainy. Roslynnyi svit* [*Red Data Book of Ukraine. Plant Kingdom*]. Ed. Ya.P. Didukh, Kyiv: Globalconsulting, pp. 803–804.

Fiasson J.L., Petersen R.H. 1973. *Carotenes in the fungus Clathrus ruber (Gasteromycetes)*. Mycologia, 65(1): 201–203.

Franceschi V.R., Loewus F.A. 1995. *Oxalate biosynthesis and function in plants and fungi*. Calcium oxalate in biological systems, pp. 113–130.

Gadd G.M., Bahri-Esfahani J., Li Q., Rhee Y.J., Wei Z., Fomina M., Liang X. 2014. *Oxalate production by fungi: significance in geomycology, biodeterioration and bioremediation*. Fungal Biology Reviews, 28: 36–55.

Heluta V.P., Zykova M.O. 2018. *Distribution of Clathrus archeri (Phallales, Basidiomycota), a fungus to be excluded from the Red Data Book of Ukraine, within the country*. Ukr. Bot. J., 75(2): 137–142.

Hosaka K., Bates S.T., Beever R.E. 2006. *Molecular phylogenetics of the gomphoid-phalloid fungi with the new subclass Phallomycetidae and two new orders*. Mycologia, 98: 949–959.

Johnson S.D., Jürgens A. 2010. *Convergent evolution of carrion and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus*. South African Journal of Botany, 76: 796–807.

Krieglsteiner G.J. 1992. *Das neue europäische Areal des Tintenfischpilzes – Clathrus archeri (Berk.) Dring*. Beiträge zur Kenntnis der Pilze Mitteleuropas, 8: 29–64.

Lomberg M.L., Solomko E.F. 2012. *Rost kul'tur makromicetov na agarizovannyh pitatel'nyh sredah i plotnyh substratah* [*Growth of the macromycetes cultures on agar nutrient media and solid substrates*]. [In:] *Biological features of medicinal macromycetes in culture*. Ed. S.P. Wasser, Alterpres, Kyiv: 345–371. [in Russian].

Lomberg M.L., Mykchaylova O.B., Bisko N.A. 2015. *Mushroom culture collection (IBK) as a subject of national heritage of Ukraine*. Ukrainian Botanical Journal, 72(1): 22–28.

Matus G., Csiky J., Bauer N. 2018. *Taxonomical and chorological notes 7*(75–84). Studia Bot. Hung, 49(2): 83–94.

Nobles M.K. 1971. *Cultural characters as a guide to the taxonomy of Polyporaceae. International Symposium Evolution in Higher Basidiomycetes*. Ed. R. Peterson, Knoxville: Univ. Tenn. Press, pp. 169–192.

Parent G.H., Thoen D., Calonge F.D. 2000. *Nouvelles données sur la réparation de Clathrus archeri, en particulier dans l'ouest et le sud-ousten de l'Europe*. Bull. Soc. Mycol. Fr., 116(3): 241–266.

Pasailiuk M.V., Petrichuk Y.V., Tsvyd N.V., Sukhomlyn M.M. 2018. *The aspects of reproduction of Clathrus archeri (Berk.) Dring by the re-situ method in the National Nature Park Hutsulshchyna*. Leśne Prace Badawcze, 79(3): 287–293.

Pearce T.R., Antonelli A., Brearley F.Q. 2020. *International collaboration between collections based institutes for halting biodiversity loss and unlocking the useful properties of plants and fungi*. Plants, People, Planet, 2(5): 515–534.

Pietras M., Rudawska M., Iszkuło G., Kujawa A., Leski T. 2016. *Distribution and molecular characterization of an alien fungus, Clathrus archeri, in Poland*. Pol. J. Environ. Stud., 25(3): 197–1204.

Prydiuk M.P., Lomberg M.L. 2021. *First record of Coprinopsis strossmayeri* (*Psathyrellaceae*) *in Ukraine: morphological and cultural features*. Czech Mycology, 73(1): 45–57.

Snetselaar K.M., Whitney K.D. 1990. *Fungal calcium oxalate in mycorrhizae of Monotropa uniflora*. Can. J. Bot. 68(3): 533–543.

Species fungorum. 2021 (online). Coordinated by the Royal Botanic Gardens, Kew. Retrieved from http://www.speciesfungorum.org/Names/Names.asp, access: 14.04.2021.

Stalpers J.A. 1978. *Identification of wood-inhabiting Aphyllophorales in pure culture*. Studies in Mycology, 16: 248.

Stebel A. 2015. *Occurrence of the fungus Clathrus archeri (Phallaceae) in the Rożnowskie Foothills (Western Carpathians, Poland)*. Acta Mus. Siles. Sci. Natur., 64: 17–19.

Stengl-Rejthar A., Wojewoda W. 1985. *Expansion of the fungus Clathrus archeri (Berk.) Dring (Gasteromycetes) in Europe and Poland*. Zeszyty Naukowe Uniwersytetu Jagielońskiego. Prace Botaniczne, 13: 105–110.

Tuason M.M., Arocena J.M. 2009. *Calcium oxalate biomineralization by Piloderma fallax in response to various levels of calcium and phosphorus*. Applied and Environmental Microbiology, 75(22): 7079–7085.

Zykova M.O. 2007. *New records of Anthurus archeri (Berk.) E. Fisch. (Clathraceae, Basidiomycota) in Ukraine*. Chornomorski Botanical Journal, 3(2): 124–128.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 299–319, Y. 2021

AQUATIC MACROPHYTE DYNAMICS OF ELEYELE LAKE, IBADAN, NIGERIA

***Yewande Adetoke Sunday*[1], *Adetola Jenyo-Oni*[2], *Emmanuel Kolawole Ajani*[3]**

[1] ORCID: 0000-0002-5333-9978
[2] ORCID: 0000-0003-2523-0614
[3] ORCID: 0000-0002-8419-0520
[1–3] Department of Aquaculture and Fisheries Management
University of Ibadan, Ibadan, Nigeria

Key words: macrophytes, floristic quality assessment, biodiversity indices, aquatic macrophyte community index, Eleyele Lake.

Abstract

This study evaluated the dynamic state of aquatic macrophytes in Eleyele Lake. Sampling was done temporally and spatially over a period of twenty-four months, covering two wet and dry seasons, in five hydrological zones (Z1–Z5) representative of the lake. Macrophytes composition was evaluated with point transect method, while data obtained were analysed using descriptive statistics. The results reveal that there were fourteen species of macrophytes present during rainy season, in contrast to seven species recorded during dry season. The value of aquatic macrophytes community index (AMCI) was higher in wet season (42) than dry season (33), although Z2, Z3 and Z4 had highest AMCI value (37). Also, results shows that the lake has moderate capacity to tolerate disturbances, low vegetative quality with increasing macrophytes coverage and decreasing open water area. Therefore, Eleyele Lake can be regarded as having diverse aquatic macrophytes population, although the biological quality is moderately poor.

Introduction

Aquatic macrophytes play a major important role in community structuring of aquatic ecosystem. It is an indicator of pollution in water quality (Cross and Mcinerny 2006, Oyedeji et al. 2013) Macrophytes have positive effects on water clarity (Boyd 1968, Peter 2000, Shekede et al. 2008). These effects include provision of habitat for plant-associated macro-invertebrates and refuge for cladocerans, both of which graze on phytoplankton and epiphytes. In lakes and reservoirs, the tropic interaction can

Address: Yewande Adetoke Sunday, University of Ibadan, Ibadan, Nigeria, phone: +234-705-489-8119, e-mail: yewaine17@gmail.com

be altered through extensive growth of aquatic plants. The population of fish that feed directly on planktonic algae could be negatively affected by dense macrophytes coverage. While aquatic macrophyte represents an important habitat for fish, excessive proliferations of these macrophytes reduce fish catch and productivity (PETER 2000).

Human activities have beneficial and adverse effects on aquatic macrophytes community. The upsurge in urban population and the production of house-hold products have resulted in increased production of household waste materials (OLADELE et al. 2018). The proximity of man to aquatic environments has made water bodies susceptible to various types of wastes from anthropogenic activities (OLADELE et al. 2019). Also the need to increase production of food due to increase in population growth has led to higher rate of production and use of agrochemicals (TETSURO et al. 2005). Influxes of domestic, industrial and agricultural wastes have been rising in both terrestrial and aquatic environments. When deposited in inland waters, such water body becomes a sink for an array of substances ranging from sewages, surface run-off, agrochemicals to industrial effluents (AROWOMOLE 1997, ADEWUYI et al. 2010, JENYO-ONI and OLADELE 2016, OLADELE et al. 2019). Direct channelling of domestic, agro and industrial wastes to inland water bodies enhances macrophytes proliferation (MUSTAPHA 2010). Wastes are sources of nutrients and pollutants, hence, it elevates the problem of waste disposal (OLADELE et al. 2018).

In Nigeria, a country where environmental safety procedures are not firmly enforced, macrophytes proliferation in inland water bodies causes waterways blockage, limited navigation through water, reduction in fishing area and fishing gear entangling and spoilage (OLOWU et al. 2010, MUSTAPHA 2010). All of these lead to reduction in the daily income of fisher folks. One of such inland waters facing the menace of macrophytes proliferation is Eleyele Lake, a freshwater lake located in South-western part of Nigeria. The operational activities of the various establishments around the lake expose it to surface run-offs and make it a destination for erosion and overland water flows. Such inflows do contain wastes from domestic, agricultural and industrial sources. `Hence, the need to assess the dynamic variation of the macrophytes in the lake becomes pertinent considering its socio-economic roles. Therefore, this study evaluated the dynamic state of aquatic macrophytes of Eleyele Lake in time and space.

Materials and Methods

Study area

The study was carried out in Eleyele Lake, an artificial lake located in Oyo State, Southwestern part of Nigeria. The Lake was built by the Water Corporation of the Old Western Region of Nigeria in 1939 (JEJE et al. 1997). Eleyele environ is surrounded by households, agricultural farm settlements, cassava-processing industries, small-scale industries such as block industry and palm kernel oil processing industry, worship centres and many others. The lake was built on River Ona, with a pool storing capability of 29.5 million litres. The dam is situated alongside Eleyele wetland in the North-eastern area of Ibadan city, precisely in Ido Local Government Area of Oyo State. The lake is located within longitude N07°25’00” and N07°27'00" and latitude E03°50'00" and E03°53"00" with a water surface area of about 162 ha.

Sampling techniques

Sampling was done based on time and space stratification over a period of 24 months. Time stratification covers two wet (May–Nov) and dry (Dec–April) seasons, based on the rainfall pattern of the environment (MUSTAPHA 2010, KAREEM 2016). Space stratification was done in line with the methods of AJANI (2001) and SUNDAY and JENYO-ONI (2018). Based on hydrological features, the lake was divided into five zones namely Oluseyi, Apapa-ijokodo, Oniyere, Dam and Igunle-ero, which were designated as zones 1 to 5 respectively. Three sampling points were randomly located in each zone. Using GPS, sampling sites coordinates were determined in each of the sampling stations. The coordinates of the sampling sites were presented in Table 1.

Table 1

Morphometric features of the selected stations in Eleyele Lake
(Sampling Stations/ Design)

Zone	Station	Co-ordinates	Maximum depth [m]	Elevation [m]
Z1	*A* – Oluseyi	07º25'32.1"N 03º51'52.4"E	6.02	180
	B – Agbaje	07º25'54.1"N 03º51'61.3"E	7.50	181
	C – Babalegba	07º25'41.2"N 03º51"80.2"E	5.31	180
Z2	*A* – Apapa-ijokodo	07º25'47.2"N 03º51"60.4"E	5.80	181
	B – Apete	07º25’59.5”N 03º51’64.3”E	6.83	180
	C – Elewure	07º25’88.4”N 03º51’88.8”E	6.00	182

cont. Table 1

Z3	*A* – Oteru	07°25’64.2”N 03°51’43.1”E	6.41	181
	B – Idi-osan	07°25’69.7”N 03°51’54.4”E	5.60	181
	C – Oniyere	07°25’95.7”N 03°51’36.6”E	6.10	181
Z4	*A* – Dam	07°25’71.3”N 03°51’32.5”E	10.40	180
	B – Spillway	07°25’82.5”N 03°51’44.9”E	11.51	180
	C – Ologuneru	07°25’72.1”N 03°51’54.2”E	9.71	181
Z5	*A* – Adedokun	07°25’52.3”N 03°51’75.1”E	9.45	179
	B – Igunleero	07°25’63.4”N 03°51’81.5”E	8.91	177
	C – Fisheries	07°25’37.7”N 03°51’54.9”E	6.50	177

Sampling method

Aquatic macrophytes composition was evaluated with point transect method using a rake-based technique of collection (THOMAZ et al. 2009). Lake survey was done from a boat moving at slow speed through all the five zones. Hook was attached to a calibrated rope of 5 m and fixed with the rake. This was tossed parallel to the side of the transect which was parallel to the shore at a depth of 0.25, 0.5 and 1 metre. The rake was released into the water, and then dragged to recover the macrophytes. The rake was tossed four times in each of the three (3) sampling points which gave a total of twelve tosses in each zone (RICK MCVOY 1991, SUNDAY and JENYO-ONI 2018). Each time the rake was retrieved from the water, the number of occurrence of the macrophytes was recorded and the samples were kept in labelled polyethylene bags lined with aluminium foil, previously cleaned and treated with 5% nitric acid and rinsed with distilled water for further analysis (ACHIONYE-NZEH and ISIMAIKAIYE 2010). Macrophytes identification was done with JOHNSON (1997), AKOBUNDU and AGYAKWA (1998) and AYENI et al. (1999).

Data analysis

The data generated from collected samples were analysed temporally and spatially using analytical tools such as Frequency of Occurrence, Aquatic Macrophyte Community Index (AMCI) and Floristic Quality Index (FQI). SPSS statistical package was used for descriptive and inferential analyses. Biodiversity indices such as Simpson’s dominance, Shannon index (H^1), Equitability (J) and Margalef index (R) were computed and data were analysed with the use of ‘PAST’ software package (HAMMER et al. 2001). In order to determine the nutrient components, the proximate composition and nutrients present in the macrophytes identified were carried out using standard techniques of AOAC (2000). Nutrients analysed for were sodium, magnesium, calcium, potassium and phosphate. Secondary data were also obtained from GIS UNIT, Geography Department, University of Ibadan to show the variation in aquatic macrophytes cover over the period of ten year (2007–2016).

Analytical Techniques for Determining Macrophytes Dynamics

Frequency of occurrence

The frequency occurrence was calculated by recording the number of times each macrophyte was present in each station each time the rake was tossed. The monthly proportion [%] of all the species, the concentration and area concealed were calculated by means of frequency of occurrence.

Aquatic macrophytes community index (AMCI)

To calculate the AMCI, seven (7) parameters were used to categorise plant communities and these parameters were given range value of 1–10. They are: Maximum depth of the plant growth, Littoral area vegetated, Simpson's diversity index, Relative frequency of submerged species, and Relative frequency of sensitive species, Taxa number, and Relative frequency of exotic species (NICHOLS et al. 2000)

Floristic quality assessment (FQA)

This method, developed by WILHELM Gerould in 1970, was used to determine the vegetative integrity area based on its plant community.

Biodiversity indices for assessing macrophytes dynamics

Various indices used include Simpson's Index, Shannon Wiener index, Equitability index and Margalef's richness index.

Simpson's index

These involve the richness and percentage (proportion) of individual macrophyte species. It takes into account the frequency of occurrence (i.e. abundance) of a particular macrophyte species in a zone and also the total number of species observed in all zones (SIMPSON 1949).

Shannon Wiener index

This index evaluates the richness and proportion of macrochyte present in each zone (Shannon and Wiener 1949).

Equitability index (*J*)

The evenness with which individual macrophyte were divided among the taxa present were recorded. Shannon diversity is divided by the logarithm of number of taxa.

Margalef's richness index

Margalef index (*d*) (Margalef 1951) was enhanced to determine the species richness.

Results

The morphometric features of the selected stations with the use of GPS are shown in Table 1. The coordinates and sampling sites in each of the zones were presented.

The temporal distribution of aquatic macrophytes in Eleyele Lake is presented in Table 2. As shown in the table, there were fourteen species of macrophytes present, out of which *Poaceae* family has 3 species; *Onagraceae* has 2 species while the other families have one species each. During the wet season, all the fourteen species of macrophytes which belong to eleven families were present. However, only seven species were present during the dry season.

Table 2

Temporal variation of aquatic macrophytes in Eleyele Lake

Scientific name	Family	Dry season	Wet season
Polygonum salicifolium Brouss. ex Willd.	*Polygonaceae*	–	xxx
Pistia stratiotes Linn.	*Araceae*	xxx	xxx
Cyperus rotundus Linn.	*Cyperaceae*	–	xxx
Luffa cylindrica (Linn.) M.J. Roem	*Cucurbitaceae*	–	xxx
Sacciolepis Africana Hubb & Snowden	*Poaceae*	–	xxx
Acroceras zizanioides (Dandy)	*Poaceae*	–	xxx
Leptochloa caerulescens Steud.	*Poaceae*	x	xxx
Ludwigia abyssinica A. Rich.	*Onagraceae*	–	xxx
Ludwigia decurrens Walter	*Onagraceae*	x	xxx
Ipomoea aquatica Forssk.	*Convolvulaceae*	xxx	xxx

cont. Table 2

Ceratophyllum demersum Linn.	*Ceratophyllaceae*	–	xxx
*Nephrolepis bisserrata (*Sw.) Schott	*Pteridophyta*	x	xxx
Nymphaea lotus Linn.	*Nymphaeaceae*	xxx	xxx
Costus afer Ker-Gauel.	*Costaceae*	x	xxx

Explanations: x – present; xxx – abundant; --- – not present

Table 3 shows the frequency of occurrence of aquatic macrophytes in Eleyele Lake. In each zone, varying number of occurrence was obtained for each of the macrophytes from which the percentage occurrence was calculated. The macrophytes with the highest percentage frequency observed during the study was *Ipomoea aquatica* with 14.06% occurrence, with percentage relative frequency of 76.67%, while *Cyperus rotundus* had the the least occurrence (4.30%) and percentage relative frequency (23.33%).

Table 3

Frequency of occurrence of aquatic macrophytes in Eleyele Lake

Species	Z1	Z2	Z3	Z4	Z5	TNO	%FO	Rel. %
Polygonum salicifolium Brouss. ex Willd.	0	6	7	8	0	21	6.42	35.00
Pistia stratiotes Linn.	6	8	7	8	5	34	10.40	56.67
Cyperus rotundus Linn.	0	5	5	4	0	14	4.30	23.33
Luffa cylindrica (Linn.) M.J. Roem	0	7	5	7	0	19	5.81	31.67
Sacciolepis Africana Hubb & Snowden	0	6	4	5	0	15	4.59	25.00
Acroceras zizanioides (Dandy)	0	7	5	6	0	18	5.50	30.00
Nephrolepis bisserrata (Sw.) *Leptochloa caerulescens* Steud.	5	4	6	5	3	23	7.03	38.33
Ludwigia abyssinica A. Rich.	0	6	5	6	0	17	5.19	28.33
Ludwigia decurrens Walter	5	3	6	4	1	19	5.81	31.67
Ipomoea aquatica Forssk.	8	10	9	10	9	46	14.06	76.67
Ceratophyllum demersum Linn.	0	6	7	8	0	21	6.42	35.00
Nephrolepis bisserrata (Sw.) Schott	7	4	2	3	6	22	6.73	36.67
Nymphaea lotus Linn.	3	10	8	5	3	29	8.87	48.33
Costus afer Ker-Gauel.	3	8	10	5	3	29	8.87	33.30
Total	–	–	–	–	–	327	100	–

Explanations: Z-Zone; TNO – total number observed; FO – frequency of occurrence; Rel. % – Relative percentage

The Aquatic Macrophyte Community Index (AMCI) in Eleyele Lake, based on time and space, is presented in Tables 4 and 5. As evident in Table 4, the AMCI value was higher in wet season (42) than dry season (33). This is obvious from the higher values of the variables obtained during the wet season. As presented in Table 5, the least AMCI value (30) was recorded in zone 1 while zones 2, 3 and 4 had the highest AMCI value (37).

Table 4

Temporal variation of aquatic macrophyte community index (AMCI) in Eleyele Lake

Category	Dry	Value	Wet	Value
Maximum depth of plant growth(m)	2.7	3	3.5	6
*Littoral area vegetated [%]	≥50	10	100	10
Submerge species (Rel %)	0	1	35	2
Simpson's diversity	0.81	1	0.90	1
Taxa number	7	3	14	7
Sensitive species	7	5	13	6
Exotic species	0	10	0	10
AMCI	–	33	–	42

*From a plant 'perspective' 100% of the littoral zone vegetated is the best

Table 5

Spatial variation of aquatic macrophyte community index (AMCI) in Eleyele Lake

Categories	Z1	Value	Z2	Value	Z3	value	Z4	Value	Z5	Value
Maximum depth of plant	2.2	3	2.5	3	2.7	3	3.2	5	2.4	3
Littoral area vegetated	40	7	≥ 50	10	≥ 50	10	40	8	45	9
Simpson's Diversity	0.9	1	0.89	1	0.89	1	0.90	1	0.90	1
Taxa number	8	3	13	6	12	6	11	5	7	3
Submerge species	0	1	25	1	20	1	35	2	0	1
Exotic species	0	10	0	10	0	10	0	10	0	10
Sensitive Species	8	5	13	6	12	6	11	6	6	5
AMCI	–	30	–	37	–	37	–	37	–	33

Floristic Quality Assessment (FQA)

Using the method of WILHEM (1970) as reported by WILHEM (1977), with an 'MC' of 4.29 and 'n' value of 14, the FQA of Eleyele lake was calculated to be 16.05. Ranking of Mean C-value ranges from low (most disturbance tolerant) with an MC of 2.0 to a high (least disturbance tolerant) of 9.5. Based on this ranking, the result of this study reveals that Eleyele Lake, with an MC of 4.29, has the capacity to tolerate disturbances moderately. Similarly, using the FQA rating of WILHEM and RERICH (2017) which reported that FQA value of 1–19 indicates low vegetative quality, 20–35 indicates high vegetative while FQA of above 35 indicates 'Natural area' quality, the result of this study shows that Eleleye Lake is of low vegetative quality.

Proximate and nutrient compositions of the macrophytes collected during the study are presented in Table 6. Having analysed all the sampled macrophytes, the highest moisture content (23.15±3.39%) was obtained in *Ipomoea aquatica* while the lowest (12.85±1.26%) was obtained in *Polygonum salicifolium*. *Polygonum salicifolium* has the maximum crude protein mean value of 7.00±0.63% while *Ceratophyllum demersum* has the minimum mean crude protein content (1.46±0.07%). Table 6 also shows that the nutrient elements had varying values among the macrophytes. The highest and lowest levels of the nutrient elements are distributed among the macrophytes.

The values of diversity indices in each zone, in space and time are presented in Tables 7 and 8 respectively. Table 7 shows that Simpson's index ranges from 0.89 to 0.90, Shannon-Weiner index ranges from 2.35 to 2.40, Margalef index ranges from 1.91 to 2.14 while equitability index ranges from 0.94 to 0.97. Also, the table shows that the index values of the zones are relatively close to each other. It is evident in Table 8 that all the indices had higher values during the wet season than the dry season.

Table 6

Mean variation and standard deviation of mineral composition in aquatic macrophytes

	Moisture	Ash	CP	CFIBRE	CFAT	C	Na	Mg	Ca	K	PO4
PS	21.85±2.48	2.76±0.5	2.06±0.18	31.58±2.30	0.22±0.11	41.58±4.26	11.60±6.22	14.56±7.29	46.95±217	13.54±4.10	5.94±3.26
AZ	18.25±0.13	2.60±0.11	2.74±0 .59	29.0±5.75	0.29±0.08	49.12±4.73	7.51±4.83	9.76±1.14	40.78±26.16	11.59±4.01	2.32±0.87
NL	23.15±2.59	1.87±0.17	2.58±0.13	23.75±4.39	0.14±0.03	43.05±3.52	14.58±0.87	34.48±2.19	32.23±1.40	18.83±0.49	10.63±0.71
CD	16.45±4.88	5.64±1.99	1.46±0.07	43.48±8.65	0.50±0.12	32.08±5.53	21.13±11.67	28.37±7.86	12.03±0.38	24.43±17.54	4.95±0.43
PS	12.85±1.26	4.50±0.44	7.0±0.63	38.68±1.67	0.38±0.03	34.6±0.96	5.0±0.22	78.03±10.39	14.6±0.77	9.7±0.88	3.25±0.28
NB	17.38±0.76	5.08±0.85	2.31±0.59	41.18±2.53	0.74±0.08	33.18±1.33	7.06±1.23	65.18±16.47	8.71±0.55	8.75±0.95	2.88±0.20
LD	20.85±0.93	2.17±0.39	2.94±0.79	30.30±2.45	0.29±0.08	43.00±1.20	4.69±0.83	89.38±9.76	32.83±13.43	11.57±3.81	3.73±1.66
LA	16.63±4.84	2.66±0.26	2.39±0.25	38.00±1.13	0.14±0.03	46.35±0.80	15.00±1.05	87.18±3.98	31.00±1.83	8.88±0.46	9.76±0.29
LCY	18.98±0.22	5.73±0.74	5.99±0.37	35.15±1.71	0.38±0.05	34.4±0.55	5.01±1.05	88.05±11.46	12.75±0.31	7.77±1.32	3.02±0.19
IA	23.15±3.39	2.73±0.37	2.46±0.24	30.08±2.69	0.25±0.11	40.28±3.32	6.76±2.71	64.7±3.38	22.20±9.43	9.11±3.18	5.43±3.33
CR	15.1±2.49	6.93±1.19	5.38±0.96	38.43±2.29	1.13±0.23	33.65±0.54	6.82±1.42	81.45±3.16	10.31±1.62	14.58±1.49	3.22±0.43
LCA	11.53±0.15	1.90±0.15	2.67±0.09	26.05±4.77	0.19±0.09	47.00±1.66	14.93±0.92	40.83±11.38	35.08±4.65	19.05±0.61	10.56±0.54
SA	13.22±0.21	1.52±0.17	2.43±0.44	22.11±2.33	0.16±0.07	32.11±1.54	7.01±0.45	42.11±6.51	18.61±0.78	17.51±0.87	8.61±0.73
CA	11.03±0.51	3.34±0.56	2.06±0.57	25.06±2.06	0.47±0.03	38.08±1.41	4.55±0.61	25.77±5.67	10.44±0.45	15.89±0.62	4.55±0.65

Explanations: horizontal axis: Cp – Crude protein; Cfibre – crude fibre; Cfat – crude fat; C – carbohydrate; Na – sodium; Mg – magnesium; Ca – calcium; K – potasssium; PO_4 – phosphate; Vertical axis: PS – *Pistia stratiotes;* AZ – *Acroceras zizanioides;* NL – *Nymphaea lotus;* CD – *Ceratophyllum demersum;* PS – *Polygonum salicifolium,* NB – *Nephrolepis biserrate;* LD – *Ludwigia decurrens;* LA – *Ludwigia abyssinica;* LCY – *Luffa cylindrica;* IA – *Ipomoea aquatica;* CR – *Cyperus rotundus;* LCA – *Leptochloa caerulescens;* SA – *Sacciolepis africana;* CA – *Costus afer*

Table 7

Spatial diversity indices of macrophytes composition

Diversity index	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Simpson 1-D	0.90	0.89	0.89	0.90	0.90
Shannon-*H*	2.40	2.35	2.35	2.36	2.36
Margalef	2.14	2.02	1.92	1.91	2.03
Equitability	0.97	0.95	0.94	0.95	0.95

Table 8

Temporal diversity indices of aquatic macrophytes

Diversity index	Wet season	Dry season
Simpson 1-D	0.90	0.81
Shannon-*H*	2.41	1.70
Margalef	1.60	0.88
Equitability	0.97	0.95

Table 9 present the variation in the spatial distribution of macrophytes and water in Eleyele Lake. Over the years under consideration (secondary data, Source: GIS UNIT, Geography Department, University of Ibadan, Nigeria), Table 9 shows that there was increase in the macrophytes coverage of the water body coupled with decreasing area of macrophytes turning to water. The table also shows that there were fluctuations in the area of water to turning to macrophytes while open water area reduced. The pictorial representation of Table 9 is presented in Figures 1, 2 and 3 respectively.

Table 9

Percentage change in spatial distribution of macrophytes of Eleyele Lake between 2007 and 2016

Aquatic Macrophytes dynamics	2007–2012 Change [%][A]	2012–2014 change [%][B]	2014–2016 change [%][C]	DF (B–A)	DF (C–B)	DF (C–A)
Macrophytes to macrophytes	23.13	32.60	48.06	9.47	15.46	24.93
Macrophytes to water	14.62	8.49	3.36	-6.13	-5.03	-11.26
Water to macrophytes	15.56	20.07	8.54	4.51	-11.53	-7.02
Water to water	46.69	38.84	39.94	-7.86	1.11	-6.75
Total	100	99.99	100	-0.01	0.01	-0.1

Explanation: DF – difference

Source: GIS Unit, Geography Department, University of Ibadan

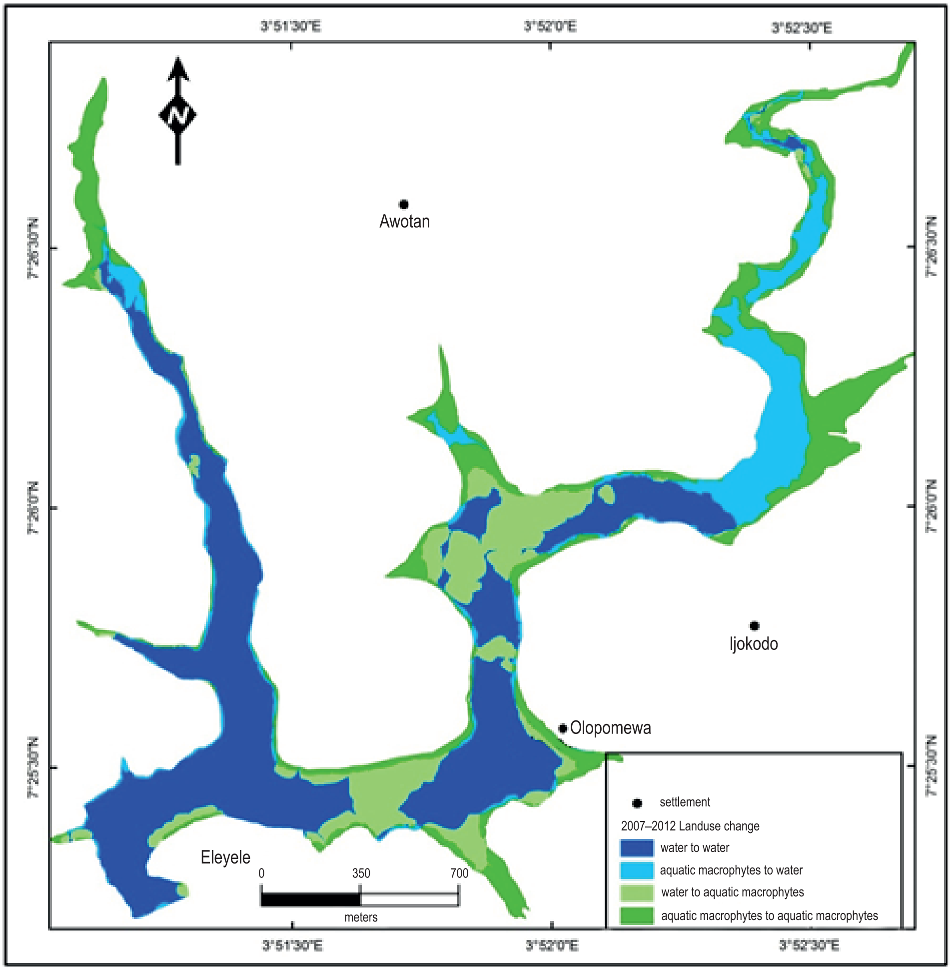


Fig. 1. Spatial distribution of macrophytes in Eleyele Lake in 2007–2012
Source: own study based on data from GIS Unit, Geography Department, University of Ibadan, Nigeria

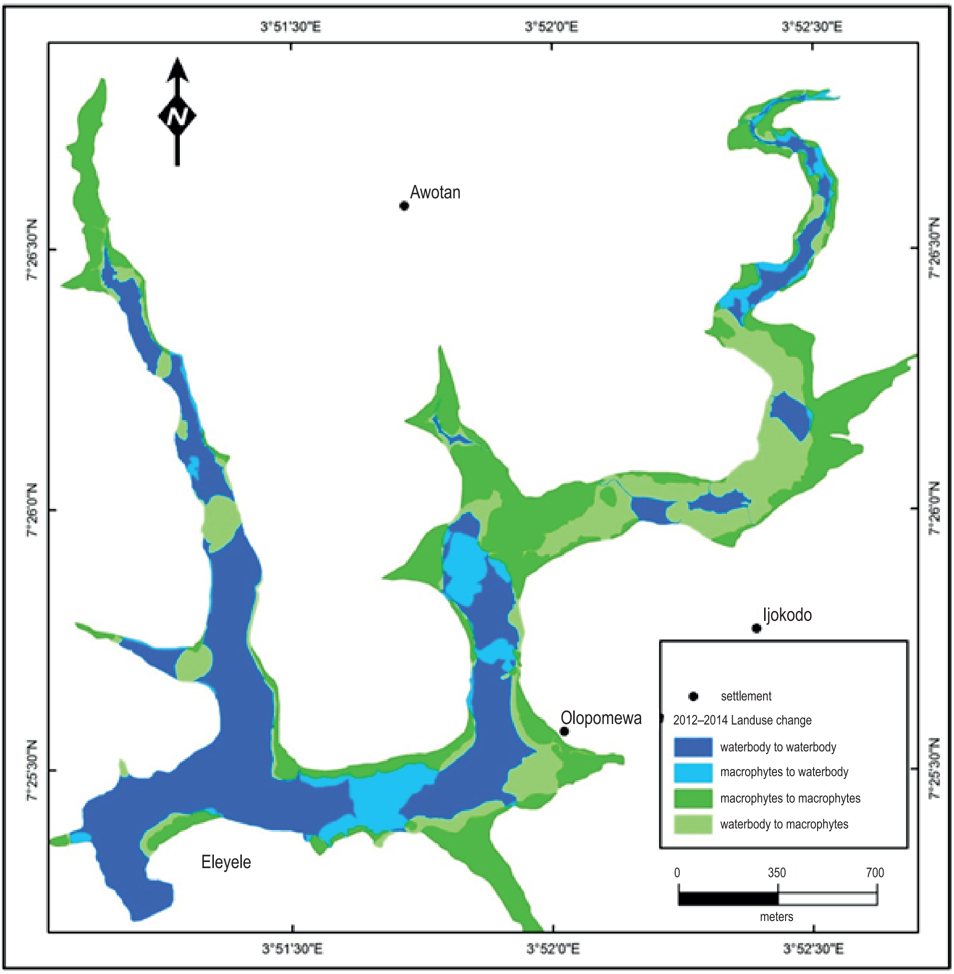


Fig. 2. Spatial distribution of macrophytes in Eleyele Lake in 2012–2014
Source: own study based on data from GIS Unit, Geography Department, University of Ibadan, Nigeria

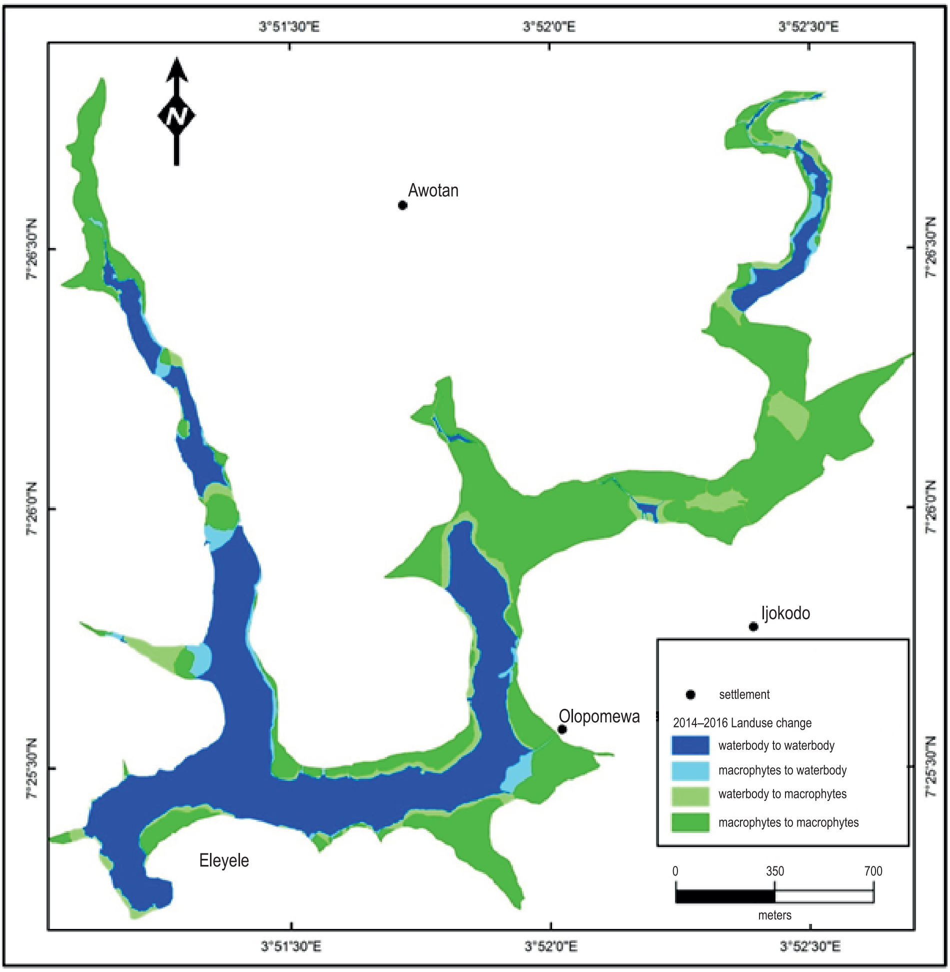


Fig. 3. Spatial distribution of macrophytes in Eleyele Lake in 2014–2016
Source: own study based on data from GIS Unit, Geography Department, University of Ibadan, Nigeria

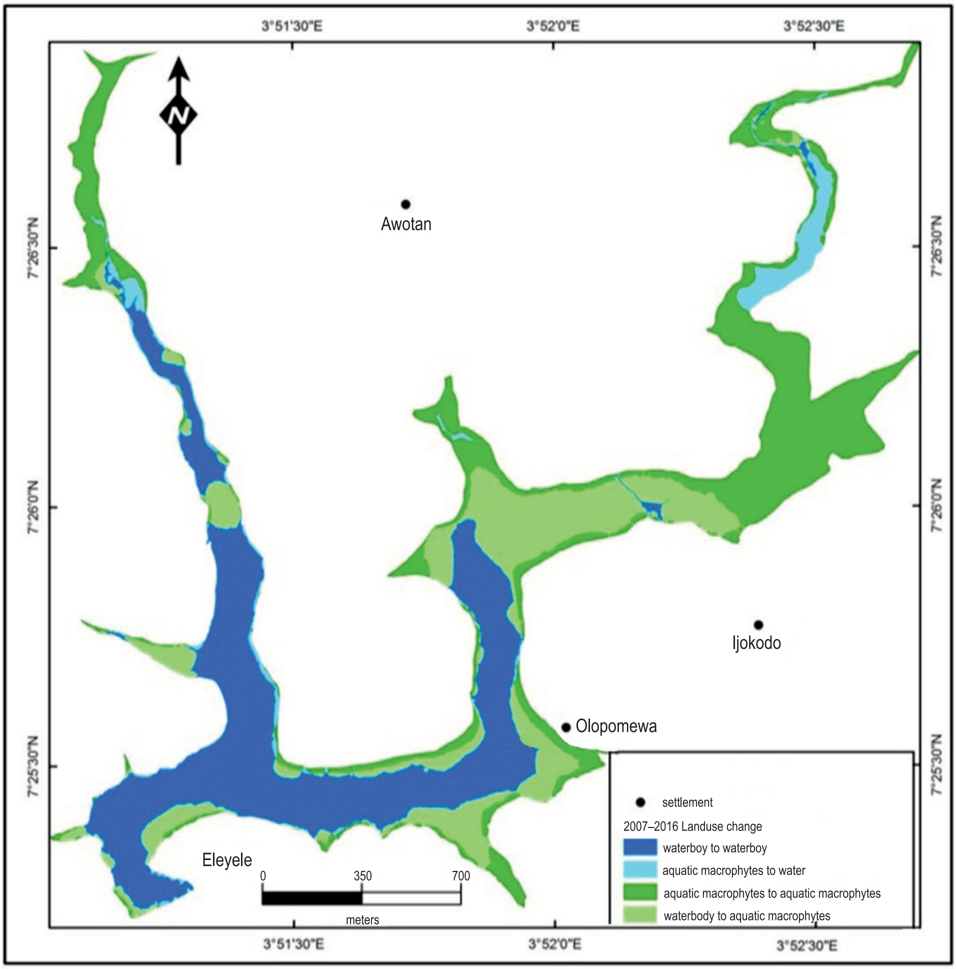


Fig. 4. Macrophyte dynamics and spatial distribution in Eleyele Lake in 2007–2016
Source: own study based on data from GIS Unit, Geography Department, University of Ibadan, Nigeria

Discussion

The composition of aquatic macrophytes identified in Eleyele lake shows that the lake has diverse aquatic plant population. Inflow of nutrient rich eroded water, which is capable of increasing the nutrient level of the water body may have played important role in the diversity observed among the macrophytes. According to the findings of PANKHURST (2005), LUND (2017), SUNDAY (2019), nutrient availability in fresh water bodies enhances aquatic macrophytes proliferation. The effect of wind and tidal action on macrophytes movement may have aided the distribution of plants in various parts of the lake. Macrophytes diversity observed in this study is relatively lower when compared with the findings of DELPHINE et al. (2015). In their report, DELPHINE et al. (2015) identified a total of 38 and 46 species of aquatic macrophytes in Gyawana and Kiri lakes, respectively. These freshwater lakes, however, are located in Adamawa State, North-eastern Nigeria. Several factors may be responsible for this variation, among which is the water volume, nutrient content, interaction among plant species, anthropogenic activities in and around the lake, among others (CONNORS et al. 2000, DAR et al. 2014, SUNDAY 2019). Also, differences in physiological characteristics of macrophytes, in addition to the effect of nutrient inflow and tidal action, may have supported the proliferation of some macrophytes over others (VERMAAT and DE BRUYNE 1993, PANKHURST 2005). This is evident in the relatively higher frequency of occurrence displayed by *Ipomoea aquatica* in the lake when compared with other macrophytes.

Temporally, the aquatic macrophyte community index (AMCI) in Eleyele Lake reflects the importance of seasonality on macrophytes population. Water addition to the lake from rainfall, erosion and the lake's feeding rivers was higher during raining season. This led to increase in water volume which in turn provides greater space for macrophytes growth (KUMAR and PANDIT 2008). The incursion of nutrient elements from these inflows provide higher nutrient base for macrophytes proliferation during the wet season. Therefore, it is not unexpected that zones 2, 3 and 4 (Apapa-ijokodo, Oniyere and Dam respectively) had the highest AMCI values among other locations. This is so because these three (3) zones are bordered with agricultural farms, industrial and residential establishments, whose wastes and effluents are deposited in the lake. Such wastes have been reported to contain nutrient elements (SUNDAY and JENYO-ONI 2018, SUNDAY 2019), thereby enhancing greater macrophytes proliferation in those zones.

However, human disturbance in form of macrophytes clearing is largely responsible for the low AMCI recorded in zone 1 (Oluseyi), since the zone is utilized for anthropogenic activities such as washing, bathing, swimming and navigation. Despite that the AMCI values obtained in this study showed that Eleyele Lake was moderately poor in biological quality, this finding is in agreement with the set limit by WEBER et al. (1995). The need for space for domestic water use, fishing and navigation has made constant clearing of macrophytes in Eleyele Lake a continuous activity. This could be responsible for the low vegetative quality of macrophytes recorded in the lake. The role of herbivorous fishes inhabiting the lake in reducing macrophytes vegetative quality cannot be overruled. According to the findings of CONNORS et al. (2000), herbivorous activities of aquatic vertebrates and invertebrates and plant interaction among other factors affect the composition and distribution of aquatic vegetation in freshwater lakes.

The availability of aquatic plants as source of food has been reported as one of the factors that enhances growth and reproduction of herbivorous fish species, especially the cichlids MUSTAPHA (2010), EDWARD (2013), OLOPADE and RUFAI (2014). The dominance of this fish species in Eleyele Lake may have played significant roles in altering the value of FQA recorded during this study. The low FQA also buttresses the primary purpose of constructing the lake, which is for the provision of potable water to Ibadan metropolis, thereby attaching little or no importance to the macrophytes composition and diversity.

Proximate composition of the aquatic plants reveals that the plants have relatively high moisture and crude fibre contents while other proximate parameters were low. This finding is in agreement with the submission of BOYD (1968) and HAZRA et al. (2018) which stated that variation in mineral composition of aquatic plants depends on plant type and age as well as the nutrient content available in the water body. However, the crude protein content observed in the aquatic plants analysed falls within the crude protein range established by the findings of BOYD (1968). All the aquatic plants were rich in minerals elements, at levels similar to the findings of BANERJEE and MATAI (1990).

This study also reveals that there were increases in the macrophytes coverage of the water body coupled with decreasing area of macrophytes turning to water. This may be attributed to inflow of nutrients into the water body (ROSSET et al. 2010, ALAHUHTA 2011, DAR et al. 2014, SUNDAY 2019). This finding was also buttressed by the fact that there were fluctuations in the area of water turning to macrophytes while open water area reduced. The decrease in the area of water distribution observed in this study may lead to reduction or loss of fish habitats. According to the submissions of TIJANI et al. (2011), reduction in the water area has negative impact on the catchment/shoreline area, in addition to degradation or loss of habitat. This finding also supports the findings of SUNDAY (2019) which stated that reduced water area in Eleyele Lake affects water navigation, reduced the recreational activities and restricted the fishing area for the fishermen. These factors reduce fish catch and adversely affect daily income of the fisher folk fishing the lake. Increase in macrophytes proliferation reduces the rate at which light penetrates the water column which in turn affects the water transparency.

Conclusion

Fourteen species of aquatic macrophytes were identified in Eleyele Lake, out of which *Poaceae* family has 3 species; *Onagraceae* has 2 species while the other families have one species each. Therefore, the lake can be referred to as having a diverse aquatic macrophytes population. Water inflow from feeder streams, nutrient addition from point and non-point sources as well as tidal action must have played important roles in enhancing macrophytes population over the years. However, the lake's macrophytes diversity is relatively low when compared with those of other freshwater lakes in Nigeria. The relative low diversity recorded during this study could be attributed to the effects of different activities on water like domestic water use, fishing, navigation and other anthropogenic activities. Among the macrophytes present, the condition of the water body must have supported greater proliferation of *Ipomoea aquatica*, as evident in the relatively higher frequency of occurrence displayed by the plant.

In Eleyele Lake, AMCI reveals that the rainy season supported greater macrophytes performance while Apapa-ijokodo, Oniyere and Dam zone had higher AMCI spatially when compared with other zones. The proximity of these zones to human interference and nutrient inflow may have accounted for the species richness observed in those zones. Despite the AMCI values obtained in this study, Eleyele Lake was moderately poor in biological quality when compared with standard rating. This may have resulted from continuous macrophytes clearing and herbivorous feeding by aquatic vertebrates and invertebrates inhabiting the water body. The low FQA value obtained in this study also corroborate the poor biological quality rating of the lake. This study also reveals that there were increases in the macrophytes coverage of the water body. This is buttressed by the decreasing area of macrophytes turning to water as well as declining open water area. In addition to reduction or loss of fish habitats, continuous macrophytes coverage of the lake will hinder such activities as domestic water use, fishing and navigation. Therefore, there is the need for regular monitoring of macrophytes proliferation on the Lake in order to ensure sustainable use of the water resources.

Accepted for print 27.10.2021

References

Achionye-Nzeh C.G., Isimaikaiye A. 2010. *Fauna and flora composition and water quality of a reservoir in Ilorin, Nigeria*. International Journal of Lakes and Rivers, 3(1): 7–15.

Adewuyi G.O., Babayemi J.O., Olabanji A.A. 2010. *Assessment of toxicity of effluent discharged into waterways by some industries in Nigeria: a case study of Ibadan*. Pacific Journal of Science and Technology, 11(2): 538–543.

Ajani E.K. 2001. *Effects of biotic and abiotic components of the habitat on fish productivity in Lagos Lagoon, Nigeria* (Ph.D thesis), Department of Wildlife and Fisheries Management, University of Ibadan.

Akobundu I.O., Agyakwa C.W. 1998. *Handbook of West African weeds*. Ibadan: IITA Ibadan 564.

Alahuhta J. 2011. *Patterns of aquatic macrophytes in the boreal region. Implications for spatial scale issues and ecological assessment* (dissertation), Acta University of Oulu A 577: 1–66.

AOAC. 2000. *Official methods of the association of analytical chemist* 15^{th} ed Edn., Washington, DC, USA.

Arowomole A. 1997. *Biodiversity study of Lagos effect of industrial effluents on the receiving Alaro State Coastal Wetlands*. MSc Project of the stream, Ibadan, Nigeria. AJEAM-RAGEE, 10: 1–13, Department of Wildlife and Fisheries Management.

Ayeni J.S.O., Obot E.A., Daddy F. 1999. *Aspects of biology, conservation and management of aquatic vascular plant resources of Nigerian Wetland based on the Kainji lake experience*. Proceedings of a Workshop on Sustainable Management and Conservation of Fisheries and Other Aquatic Resources of Lake Chad and Arid Zone of Nigeria, Jan. 16–17, Maiduguri, pp. 64–73.

Banerjee A., Matai S. 1990. *Composition of Indian aquatic plants in relation to utilization as animal forage*. Journal of Aquatic Plant Management, 29: 69–73.

Boyd C.E., 1968. *Freshwater plants. A potential source of protein*. Economic Botany, 22: 356.

Connors L.M., Kiviat E., Groffman P.M., Ostfeld R. 2000. *Muskrat (Ondatra zibethicus) disturbance to vegetation and potential net nitrogen mineralization and nitrification rates in a freshwater tidal marsh*. The American Midland Naturalist Journal, 143:53–63.

Cross T.K., Mcinerny M.C. 2006. *Relationships between aquatic plant cover and fish populations based on Minnesota Lake survey data*. Minnesota Department of Natural Resources, Division of Fish and Wildlife Investigational Report 537, St. Paul.

Dar N.A., Pandit A.K., Ganai B.A. 2014. *Factors affecting the distribution patterns of aquatic macrophytes*. Limnological Review, 14(2): 75 –81.

Delphine D.L., Daniel A.B., Augustine E.U., Sunday O.J., Thelma A. 2015. *Biodiversity and productivity of two lacustrine wetlands of the upper Benue River Basin, Adamawa State, Nigeria*. International Journal of Aquatic Science, 6(2): 60–75.

Edward J.B., 2013. *Evaluation of the fisheries potentials of Egbe Reservoir, Ekiti State, Nigeria*. Greener Journal of Biological Sciences, 3(7): 260–267.

Hammer Ø, Harper Dat., Ryan P.D. 2001. *Past. palaeontogical statistics software packages for education and data analysis*. Palaeontologia Electronica, 4(1): 9.

Hazra H., Alfasane M.D., Kauser S., Shahjadee U.T., Khondker M. 2018. *Biochemical composition of some selected aquatic macrophytes under ex-situ conditions*. Journal of Asiatic Society of Bangladesh, Science 44(1): 53–60.

Jeje C.Y., Onogbosele C.O., Aladeojebi E.A. 1997. *An appraisal of the current status of the fisheries of eleyele resevoir by its fisheries cooperatives*. Department of Zoology, University of Ibadan, Nigeria.

Jenyo-Oni A., Oladele A.H. 2016. *Heavy metals assessment in water, sediment and selected Aquatic organisms in Lake, Asejire, Nigeria*. European Scientific Journal, 12: 24:1857.

Johnson D.E. 1997. *Weeds of rice in West Africa,* AVRAO/WAKDA, Cote d'lvoire, p. 312.

Kareem O.K. 2016. *Ecological evaluation and sustainable fish production in Erelu reservoir, Oyo, Nigeria*. PhD thesis submitted to Department of Aquaculture and Fisheries Management.

Kumar R., Pandit A.K. 2008. *Effect of water level fluctuations on distribution of emergent vegetation in Hokerser wetland, Kashmir*, Proceedings of the National Academy of Science, India, Section B, 78: 227–233.

Lund K. 2017. *Lake George, Anoka County 2016. Aquatic vegetation report. report by the invasive species program*. Division of Ecological and water resources Minnesota, Department of Natural Resources. Management, 24: 1–10.

Margalef R. 1951. *Diversidad de species en las commundales naturals.* Publicaciones del Instituto de Biologia Aplicada, 6: 59–72.

Mustapha M.K.. 2010. *Fish fauna of Oyun Reservoir, Offa, Nigeria.* Journal of Aquatic Sciences, 25(1): 106–114.

Nichols S.A., Weber S., Shaw B. 2000. *A proposed aquatic plant community biotic index for Wisconsin lakes.* Environmental Management, 26, 491–502.

Oladele A.H., Digun-Aweto O. Sunday Y.A 2019. *Relationship between cadmium, nickel and chromium contents in Oreochromis niloticus, Macrobrachium vollenhovenii, sediments and water of Lake Asejire, Nigeria.* World Journal of Fish and Marine Sciences, 11(2): 15–22.

Oladele A.H., Digun-Aweto O., Jenyo-Oni A. 2018. *The relationship between iron, lead and cobalt content in water, sediments, Nile tilapia and African River Prawn of lake Asejire, Nigeria.* Polish Journal of Natural Sciences, 33(3): 439–454.

Olopade O.A., Rufai O.P. 2014. *Composition, abundance and diversity of the Family Cichlidae in Oyan Dam, Ogun State, Nigeria.* Biodiversitas, Journal of Biological Diversity, 15(2): 195–199.

Olowu R.A., Ayejuyo O.O., Adewuyi G.O., Adejoro I.A., Denloye A.A.B., Babatunde A.O, Ogundajo A.L. 2010. *Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria.* E-Journal of Chemistry, http://www.e-journals.net, 7(1): 215–221.

Oyedeji S., Fatoba P.O., Ogunkunle C.O., Akanbi G.M. 2013. *Water hyacinth and duckweed as indicator of heavy metal pollution in River Asa* Journal of Industrial Pollution Control., 29(2): 155–162

Pankhurst H. 2005. *Patterns in the distribution of aquatic macrophytes in Georgian Bay, Ontario*. Final Report for Senior Honours Project Submitted to the Department of Biology McMaster University 1280 Main Street West. Hamilton, ON L8S 4K1.

Peter T. 2000. *Interactions between fish and aquatic macrophytes in inland waters.* Fisheries Technical Paper, An FAO review, No. 396.

Rick Mc Voy. 1991. *Aquatic plant identification and monitoring assistance*. A methods manual EPA 440/4-91-002. Department of Environmental Protection, Division of Watershed Management, Worcester.

Rosset V., Lehmann A., Oertli B. 2010, *Warmer and richer? Predicting the impact of climate warming on species richness in small temperate water bodies*. Russian Journal of Plant Physiology, 51: 21–27.

Shannon C.E, Wiener W. 1949. *The mathematical theory of communication urban*. University of Illinois Press.

SHEKEDE M.D., Kusangaya S., Schmidt K. 2008. *Spatio-temporal variations of aquatic weeds abundance and coverage in Lake Chivero, Zimbabwe.* Physics and Chemistry of the Earth, 33: 714–721.

Simpson W. 1949. *Measurement of diversity*. Journal of Natural Hist., 163: 688.

Sunday Y.A 2019. *Macrophytess dynamics and its impact on fish productivity in Eleyele Lake.* Ph.D. thesis, University of Ibadan, Nigeria.

Sunday Y.A., Jenyo-Oni A. 2018. *Spatial and temporal variation of limnological features of Eleyele Lake, Ibadan, Nigeria.* O.A Fagbenro et al. (eds). Proceedings of 1st Conference and Annual general meeting "Science, Innovation and Aquabusiness", University of Ibadan, Ibadan, Nigeria. July 10–12, 2018, pp. 263–270.

Thomaz S.M, Carvalho P., Padia A.A., Kobayashi J.T. 2009. *Temporal and spatial patterns of aquatic macrophyte diversity in the Upper Parana River flood plain.* Brazilian Journal of Biology, 69: 6ll–625.

TIJANI M.N., OLAEYE A.O., OLUBANJO O.O. 2011. *Impact of urbanization on wetland degradations case study of Eleyele Wetland, Ibadan, South West, Nigeria*. Proceedings of the Environmental Management Conference, Federal University of Agriculture, Abeokuta, Nigeria, pp. 435–457.

VERMAAT J.E., DE BRUYNE R.J. 1993. *Factors limiting the distribution of submerged waterplants in the lowland River Vecht* .The Netherlands, Freshwater Biology, 30: 147–157.

WEBER S., SHAW B., NICHOLS S. 1995. *The aquatic plant community of Eight Northern Wisconsin Flowages.* University of Wisconsin-Stevens Point. Stevens Point, WI.

WILHELM G., RERICH L. 2017. *Flora of the Chicago Region. A floristic and ecological synthesis*. Indianapolis, Indiana Academy of Science.

WILHELM G. 1977. *Ecological assessment of open land areas in Kane County Illinois*. Kane County Urban Development Division, Geneva Illinois.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 321–332, Y. 2021

ANTIBACTERIAL ACTIVITIES AND SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM FISH MUCUS TO SELECTED CLINICAL BACTERIA ISOLATES

***Abidemi Esther Ojo*[1], *Thaddeus O. Ariom*[2], *Olugbenga Anthony Ojo*[3], *Seun Owolabi Adebajo*[4], *Abiola Tosin Oladotun*[5], *Sherifat Arike Opaleye*[6], *Nimotallahi Rabiu*[7]**

[1] ORCID: 0000-0001-6359-4086
[2] ORCID: 0000-0002-2959-8507
[4] ORCID: 0000-0001-7981-2965
[5] ORCID: 0000-0002-4592-7585
[6] ORCID: 0000-0002-1885-9759
[7] ORCID: 0000-0001-5508-1280
[1,2,4–7] Department of Microbiology
Federal University of Agriculture, Abeokuta, Nigeria
[3] Department of Pharmacognosy
Madonna University, Elele, Port-harcourt, River-state, Nigeria

Key words: fish mucus, antibacterial potentials, susceptibility pattern, spot-on-lawn assay and resistance.

Abstract

Fish mucus is known for its antimicrobial activities offering protection to fishes from environmental pathogenic attack. This study evaluates the antibacterial potentials of bacteria isolated from fish mucus of *Chrysichthys nigrodigitatus* and *Solea solea* on four clinical isolates: *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus* and *Aeromonas hydrophila* using the spot-on-lawn method and modified disc diffusion method. Susceptibility pattern of fish mucus bacterial isolates was determined using disc diffusion method. *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Citrobacter freundii* and *Shigella sonnei* were the isolates from the fish mucus varieties. Using spot-on-lawn assay, *Proteus mirabilis* exhibited the highest antibacterial activities against *Bacillus subtilis* (4.00±1.73 mm) while on the modified disc diffusion assay, *Shigella sonnei* exhibited highest antibacterial activities of (7.00±0.21 mm) against *Aeromonas hydrophila*. Among the isolates, only *Klebsiella pneumoniae s*hown resistance to most of the conventional antibiotics used. Hence, bacteria isolated from fish mucus possess some antibacterial properties against clinical bacterial isolates.

Address: Ojo Abidemi Esther – Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria. Email address: ojoae@funaab.edu.ng

Introduction

Fish is considered an efficient source of important nutritive protein that nourishes the body and hence consumed globally for this health benefits. Of all diet with a large reasonable amount of protein, fish stands out with closely 16% (world's population) animal protein (FAO 2007) and 17% in Africa population (ALLISON 2009). Microbiologically, fish and its bye products are of potential health risk due to its ability to harbour important human pathogenic bacteria. Bacterial infections can occur due to unhygienic handling and eating of poorly prepared ready-to-eat fish. Numerous bacterial genera such as *Escherichia*, *Listeria*, *Pseudomonas*, *Klebsiella*, and *Salmonella* have been identified from fish indicating several means of contamination of fish environs (MANHONDO et al. 2018, SICHEWO et al. 2014).

Fishes are naturally endowed with high immunological tolerance. One of the major line of defense exhibited in fishes is the skin slime that serves as first body soldiers (VENNILA et al. 2011). The mucus in the skin of fish naturally prevents invasion of most pathogenic microbes (includingand fungus) into the body of the fishes (VENNILA et al. 2011). Mucus of the fish is extensively released by the goblet cells (present in the skin) and it exclusively consists of mucins and other substances like inorganic salts, immunoglobulin, proteins and lipids (TYOR and KUMARI 2016). The mucus of *Claris* spp. have been explore in ages for its use in orthodox medicine to rejuvenate wounds, burns and tumor (CHINWUBA et al. 2016, DESLOUCHES and DI 2017). While *Anguiil abengalensis* has been in continuous use for the therapy of anaemia, burn injury, piles, and weakness in the Indian traditional medicine (RAHMAN et al. 2014), C*hanna striatus* is popular due to its ability to speed the rejuvenating rate of wounds, build up immune system and anti-inflammation including tender antifungal and antibacterial roles (WEI et al. 2010). These good roles have closely been associated to the presence of antimicrobial peptides (AMPs), polyunsaturated fatty acids (PUFA), mycosporine-like amino acids (MAAs) and organic acids (NWABUEZE and CAMPUS 2014).

Global daily increase in antimicrobial resistance to most commonly used antibiotics necessitated further attempts to search for novel antimicrobial agents preferably, non-synthetic agents, to combat infections. In 2017, WHO cries out for an emergency aid in research and development towards a world's health immediate need as there is an alarming decline rate of antibiotics particularly, the ESKAPE pathogens. If care is not taken in this century, the infections caused by these pathogens may return the world back to orthodox ages (WHO 2017). Hence the need to investigate the antibacterial properties of bacteria isolated from fish mucus.

Materials And Methods

Mucus collection and preparation for antibacterial activities

Five (5) healthy live samples each of two different fishes (*Chrysichthys nigrodigitatus and Solea solea*) regardless of age and sex were purchased from riverine area in badagry, Lagos, Nigeria. The fish samples were collected into sterile perforated container filled with water and labeled accordingly. Mucus collection was done by starving the fishes for one day without prior application of anesthesia and transported to the laboratory for microbiological analysis observing the modified method of SUBRAMANIAN et al. (2007). Briefly, Whatman no 1 filter paper was used to remove the mucus from the fishes by gently moving it from the head of the fish to the tail in order to slough off the fish mucus. This was done separately for each fish specie. Spam and intestinal cross-contamination were prevented by avoiding mucus collection through the ventral side. The aseptically collected mucus sample were mixed well with equal quantity of sterilized physiological saline (0.85% NaCl) in readiness for microbial isolation and some parts of the mucus was centrifuged at 5,000 rpm for 15 mins. The supernatant was placed in vials and kept at 4°C for future antimicrobial studies.

Acquisition and conservation of pathogenic bacterial strains

The pathogenic bacterial strains were obtained from the Federal Institute of Industrial Research (FIIRO), Lagos, Nigeria. Antibacterial activities of fish skin mucus extracts was tested against four pathogenic bacteria which includes *Escherichia coli, (*ATCC 25922*), Staphylococcus aureus,* (ATCC 25923*) and Bacillus subtilis (ATCC 6633)* and a common gram negative fish pathogenic bacteria *Aeromonas hydrophilla (ATCC 35654).* All the bacterial strains were maintained by growth in nutrient broth observing a standard biomedical safety procedures and conditions. A 10 ml of prepared nutrient broth was poured in a flask and one loop of each targeted test bacteria was added to the flask and incubated at 37°C for 24 hrs.

Isolation and characterization of associated bacteria

The method of isolation was by culture method. This was done by preparing media such as Nutrient agar (Oxoid, UK), MacConkey agar (Oxoid, UK) and Mueller Hinton agar (Oxoid, UK) following manufactures' specification. This was followed by aseptically inoculating the serially diluted mucus on the media plate of Nutrient agar (Oxoid, UK), blood agar (Oxoid, UK), mannitol salt agar (Oxoid, UK) and MacConkey agar (Oxoid, UK). The inoculated plates were labeled appropriately and incubated at a room temperature of 37°C for 24 hrs. The microbial isolates were sub cultured until pure culture were obtained before transferring to an agar slant for further use. The associated bacteria isolates were identified morphologically (size, form, colour, consistency, edges, elevation and opacity). They were further identified using Gram staining. Biochemical characterization such as indole, oxidase, voges proskauer, triple sugar iron, urease, hydrogen sulfide production test for solubilizing bacteria citrate, catalase and motility test following Bergey’s manual of systematic bacteriology (Holt et al. 1994).

Antibiotics sensitivity test

Antibiotic susceptibility test was achieved by Kirby Bauer Disc diffusion susceptibility method according to Clinical and laboratory standard institute (CLSI 2009). A Mueller-Hinton agar plate was prepared according to manufacture[r]’s specification. Three to five well-isolated colonies of the test bacteria were selected from an agar plate and suspended in 5 ml of nutrient broth. The turbidity of the broth culture was adjusted to 0.5 McFarland standard. Standardized inoculums of the organism were swabbed on the entire Mueller-Hinton agar plate within 15 minutes. At about three minutes thereafter, paper discs impregnated with the following antimicrobial agents; Augmentin (AUG) 30 µg, Chloramphenicol (CH) 30 µg, Septrin (SXT), 30 µg, Gentamycin (CN) 10 µg, Streptomycin (STR) 30 µg, Amoxacillin (AMX) 30 µg, Sparfloxacillin (SP) 30 µg, Ciprofloxacin (CPX) 30 µg, Pefloxacin (PEF) 30 µg, Tarivid (OFX) 10 µg (all from Oxiod UK) were positioned on the agar plate using sterile forceps. These plates were left for about 30 mins for predifussion to take place before incubation. The plates were incubated at 37°C for 24 hours. The diameter of zone of inhibition was measured to nearest millimeter.

Screening for antibacterial activity of organisms isolated from fish mucus

The bacterial isolates from the fish mucus were tested against pathogenic bacterial strains for their antibacterial potency using the spot on lawn assay and disc diffusion method.

The spot-on lawn antimicrobial assay used had little modification. Briefly, Mueller Hinton agar was prepared and at molten state around 40°C, the pathogenic organisms were evenly mixed with the agar and poured into the plate. Varied aliquots diluent of the test organisms are dripped onto the media that has been seeded with the pathogenic microorganisms. After aerobic incubation at 37°C for 24 hours, the antimicrobial activity was measured and expressed as inhibition zone diameter.

In disc diffusion assay, discs of 6 mm were absorbed with supernatant and placed on the agar seeded with pathogenic bacteria strains. After overnight incubation at 37°C, the inhibition zone is evaluated in millimeter (mm) based on the clear zone around the paper disc according to SOOMRO et al. (2007).

Data analysis

The data obtained were first subjected to descriptive statistics (mean and standard error of the mean). Analysis of Variance (ANOVA) was used to determine significant differences in mean across all the groups. *P*-values of less than 0.05 were considered as significant using SPSS software.

Result and discussion

Study in this research generates an insight into the antibacterial activities and antibiotics susceptibility pattern of bacteria isolated from fish mucus on clinical isolates using spot on lawn and disc diffusion method. In this case, a total of 30 bacterial isolates were identified; *Salmonella typhi* (40%), *Proteus mirabilis* (8%), *Klebsiella pneumoniae* (20%), *Shigella sonnei* (20%), and *Citrobacter freundii* (12%) – Figure 1. The bacteria identified through biochemical tests are *Klebsiella pneumoniae, Salmonella typhi, Proteus mirabilis, Shigella sonnei, Citrobacter freundii* (Table 1).

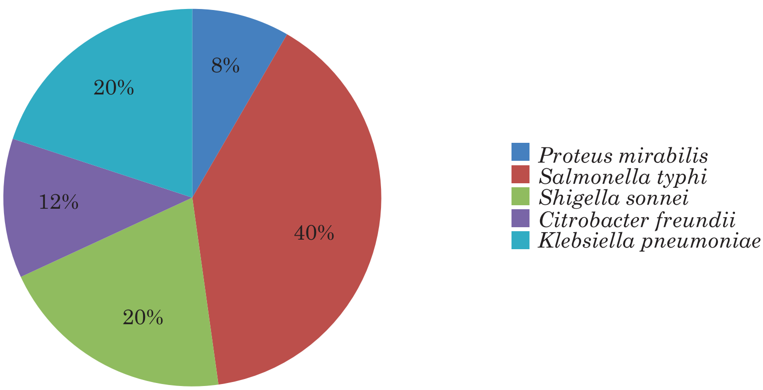


Fig. 1. Percentage occurrence of bacteria isolated from fish mucus

Table 1

Biochemical identification of bacteria isolated from fish mucus

S/N	ORG	GR	IN	OX	MO	CA	UR	CI	H_2S	TSI		
										SU	LA	GL
1.	*Klebsiella pneumoniae*	–	–	–	+	+	+	+	–	+	+	+
2.	*Proteus mirabilis*	–	–	–	–	+	+	+	+	–	–	+
3.	*Shigella sonnei*	–	+	–	–	+	–	–	–	–	–	+
4.	*Citrobacter freundii*	–	–	–	+	+	–	+	+	–	–	+
5.	*Salmonella typhi*	–	–	–	+	+	–	–	+	–	–	+

Explanations: ORG – organism, GR – gram reaction, IN – indole, OX – oxidase, CA – catalase, UR – urease, CI – citrate utization, GL – glucose, H_2S – hydrogen sulphide, TSI – triple sugar iron, SU – sucrose, LA – lactose

The fact that, *Salmonella typhi* was the most prevalent bacterial specie contradict the report of MANHONDO et al. (2018) who isolated a different set of bacteria sp. from fish in Zimbabwe. In the same vein, our results also contradicts that of HAFSAT et al. (2015) who reported *Staphylococcus aureus, Streptococcus* spp. *and Escherichia coli* as the most frequent bacterial species in the epidermal layer of fish. The difference in the bacteria species recorded may be due to seasonal variation, geographical location as well as sources of indiscriminate disposal of refuse in water bodies (CABRAL 2010).

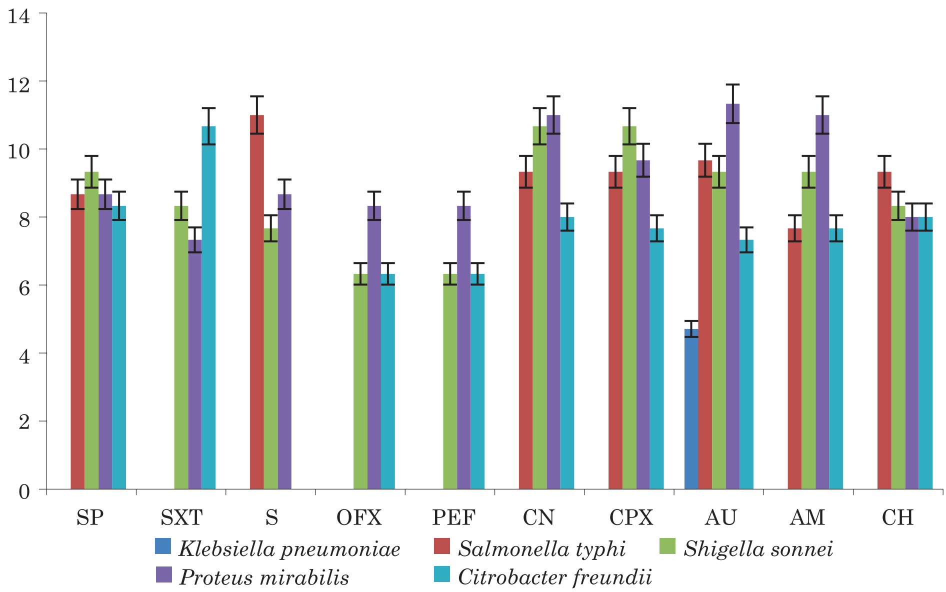


Fig. 2. Graphical representation of antibacterial activity of selected antibiotics on fish mucus isolates: SP (Sparfloxacin 10 µg), SXT (Septrin 30 µg), S (Streptomycin 30 µg) OFX (Tarivid 10 µg), PEF (Pefloxacin 30 µg), CN (Gentamycin 30 µg), CPX (Ciprofloxacin 30 µg), AU (Augmentin 10 µg), AM (Amoxacilllin 30 µg), CH (Chloramphenicol 30 µg)

The antibiotics sensitivity test of the fish mucus isolates as depicted in Figure 2 revealed that *Klebsiella pneumoniae* is resistance to virtually all the ten different antibiotics used except Augmentin (4.71±1.21). In contrast, *S. typhi* and *S. sonnei* were susceptible to all antibiotics used while *P. mirabilis* is susceptible to most of the antibiotics but resistant to three (pefloxacin, septrin and arivid). Furthermore, *C. freundii* showed resistance to only Streptomycin while being susceptible to the rest of the antibiotics. This result corroborates with the study of MANHONDO et al. (2018). They identified bacteria that were multi resistant to different antibiotics used with the exemption of *P. mirabilis*. Non-susceptibility of some of the identified isolates to antibiotics may be attributed to the presence of non-biodegradable metal disposal into the water bodies, hence, accelerating the natural selection in water bodies, enhancing the movement of genes coding for resistance of antibiotics among water loving bacteria (ALANIS 2005, SEILER and BERENDONK 2012).The antibacterial activities of fish mucus isolate *P. mirabilis* against the clinical isolates using overlay antimicrobial assay inhibited *E. coli*, *B. subtilis* and *A. hydrophila* with a zone of inhibition 2.67±1.15 mm, 4.00±1.73 mm and 1.00±1.00 mm respectively. In the disc diffusion method, *P. mirabilis* inhibited *E. coli* (3.00±1.48 mm), *B. subtilis* (5.00±2.31 mm), *A. hydrophila* (4.00±2.11 mm) – Figure 3. This result is in

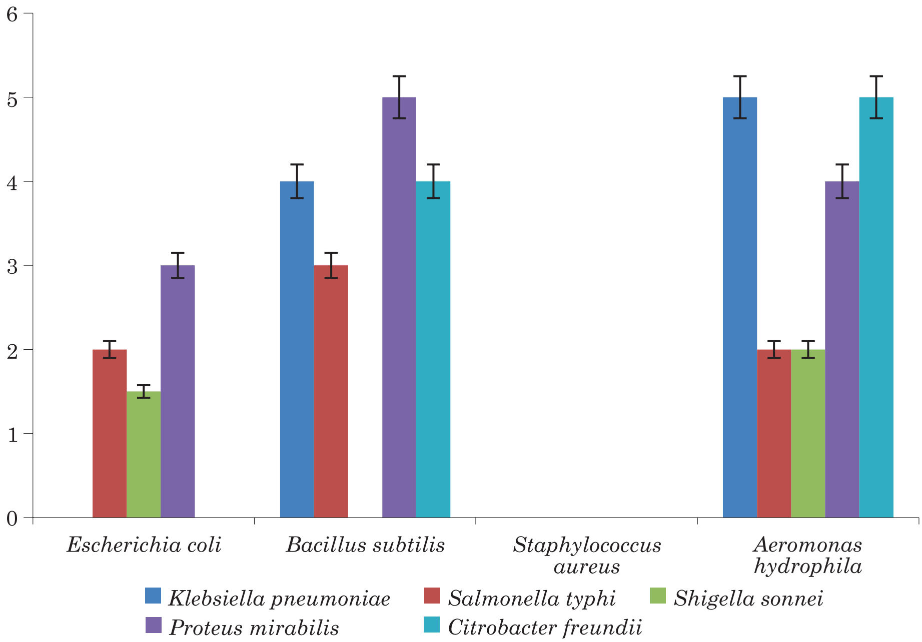


Fig. 3. Graphical representation of antibacterial activity of fish mucus isolates on clinical isolates using spot on lawn/overlay assay

agreement with the study of NAZ and RASOOL, (2013), who reported that *P. mirabilis* produces antibacterial proteins that are capable of inhibiting other organisms. *C. freundii* using spot on lawn inhibited *E. coli* (3.67±0.58 mm) and *B. subtilis* (3.33±1.53 mm). However, it showed no significant effect on *Staph. aureus* (0.00±0.00mm) and *A. hydrophila* (0.00±0.00 mm) in the overlay antimicrobial assay.

Contrastingly, *C. freundi* have no antibacterial effect on *E. coli* and *B. subtilis* but it inhibited *B. subtilis* (4.00±2.74 mm) and *A. hydrophila* (5.00±2.61 mm) in the disc diffusion assay (Figure 4). Our finding is in line with the study of ROBERT et al. (2012), who reported the production of antimicrobial compound by *C. freundii*. In this study, *Klebsiella pneumoniae* antimicrobial assay using overlay method showed inhibition on *B. subtilis* (3.67±2.8 mm), *S. aureus* (0.67±0.58 mm) and *A. hydrophila* (2.33±1.15 mm) while only *B. subtilis* (4.00±2.11 mm) and *A. hydrophila* (5.00±2.31 mm) were inhibited when disc diffusion method is used.

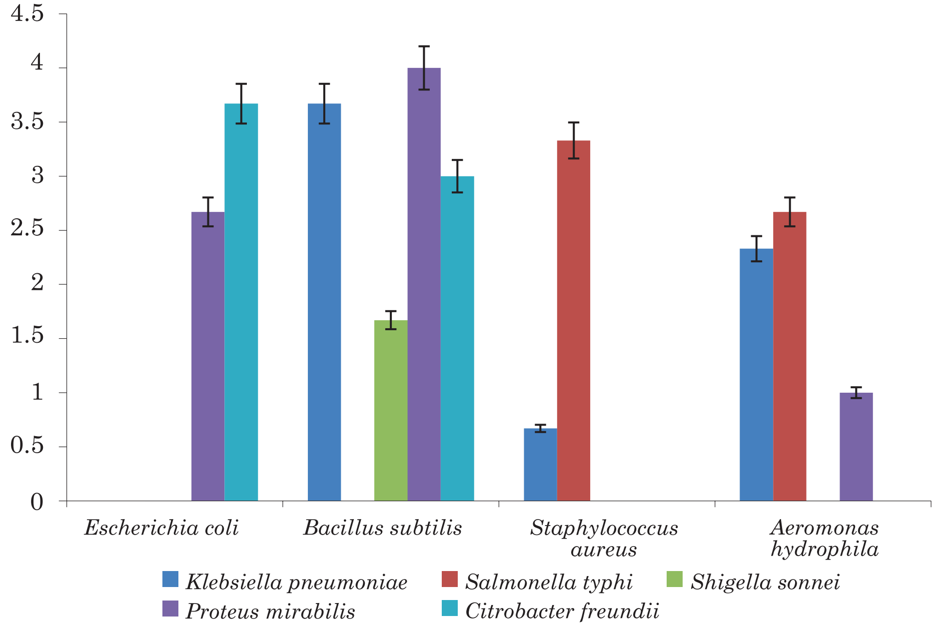


Fig. 4. Graphical representation of antibacterial activity of fish mucus isolates on clinical isolates using disc diffusion method

S. typhi inhibited only *S. aureus* (3.33±0.58 mm) and *A. hydrophila* (2.67±1.151 mm) using overlay assay, but inhibited more pathogenic organisms (*E. coli* (2.00±1.30 mm), *B. subtilis* (3.00±1.31 mm), *A. hydrophila* (2.00±1.34 mm)) when disc diffusion method was employed. *S. sonnei* inhibited only *B. subitilis* (1.67±2.08 mm) when overlay assay was used.

However, it inhibited *E. coli* (1.50±0.54 mm) and *A. hydrophila* (7.00±0.21) using disc diffusion method (Figure 4). This implies that *S. sonnei* demonstrated the least antimicrobial effect among the fish mucus isolates while *P. mirabilis* demonstrated the highest antibacterial effect.

In contrast*, Staph. aureus* showed a very high resistance to the antibacterial activity of fish mucus isolates. The inhibitory effects of the organisms isolated from fish mucus as reported in this study is in conformity with previous reports (MIDHUN et al. 2017a,b). The inhibitory action exhibited by the bacteria spp. were attributed to mass or holistic secretion of antagonistic materials which include bacteriocins, siderophores, antibiotics (SEBASTIAN et al. 2018), and lysosomal enzymes (RAY et al. 2012).

Previous studies have also indicated that, environmental isolates of *Bacillus* spp. produced bacteriocin like peptides such as lichenicidin and lantibiotic, which gave 100% resistant to the growth of both gram-negative and gram-positive bacteria (MUKHERJEE et al. 2016, SEBASTIAN et al. 2018). Similarly, another research conducted by SEBASTIAN et al. (2018) on inhibitory properties of *Bacillus coagulans* found in gut of fishes against pathogens reported that *B. coagulans* may protect the fish from invading pathogenic bacterial cells. (SEBASTIAN et al. 2018). This study further agrees with the conclusion of a study carried out in corals on antibiotic activity in fish which stated that close to a quarter of bacteria cultured from the mucus of the elkhorn coral, *Acropora palmata*, exhibited antagonistic properties against a range of pathogenic test strains whereby 8% were directly active against a causative agent of a disease in the particular species (RITCHIE 2006).

Conclusion

These results confirm that the protection found in fish against surrounding intruders could have been acquired from their epidermal slime layers. Hence, harnessing fish mucus for its antibacterial properties will not be out of place. Major limitation to the study is the fact that only two species of fishes were used in this study, hence we can't generalize antibacterial potential of fish mucus.

Translated by dr. Ojo Abidemi

Accepted for print 19.08.2021

References

ALANIS A.J. 2005. *Resistance to antibiotics: are we in the postantibiotic era?* Archives of Medical Research, 36(6): 697–705.

ALLISON E.A., PERRY A.L., BADJECK M.C. 2009. *Vulnerability of national economies to the impacts of climate change on fisheries.* Fish and Fisheries, 10(2): 173–196.

CABRAL J.P.S. 2010. *Water microbiology. Bacterial pathogens and water. International Journal of Environmental Research and Public Health,* 7(10): 3657–3703.

CHINWUBA T., OKAFOR S.N., OKECHUKWU D.C. 2016. *Catfish (Clarias gariepinus) slime coat possesses antimicrobial and wound healing activities.* UK Journal of Pharmaceutical and Biosciences, 4(3): 84–7.

Clinical And Laboratory Standards Institute (CLSI) 2009. *Performance Standards for antimicrobial susceptibility testing, 19th information supplement*, CLSI/NCCLS Document M100-s19, Wayne, PA, USA.

DESLOUCHES B., DI Y.P. 2017. *Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications.* Oncotarget, 8(28): 46 635–46 651.

FAO 2007. *Social issues in small scale fisheries: how a human rights perspective can contribute to respective fisheries in Proceedings of the Presentation to FAO* Committee of Fisheries no 27, Rome, Italy.

HAFSAT A.G., YAQUB A.G., ABUBAKAR S., ISA A.G., ROY B.B. 2015. *Multi drug resistant bacteria isolated from fish and fish hanlers in Maiduguri.* Nigeria International Journal of Animal and Ventinary Advances, 7(3): 49–54, doi: 10.19026/ijava.7.5240.

HOLT J.G., KRIEG N.R., SHEATH P.H.A., STALEY J.T., WILLIAMS S.T. 1994 *Bergey's manual of determinative bacteriology* (9th edition). *Baltimore, Maryland, USA, Williams & Wilkins.*

MANHONDO P., GONO R. K., MUZONDIWA J., MAFA B. 2018. *Isolation and identification of pathogenic bacteria in edible fish: a case study of bacterial resistance to antibiotics at Lake Chivero, Zimbabwe,* African Journal of Agricultural Research (in press).

MIDHUN S.J., NEETHU S., VYSAKH A. 2017a. *Antibacterial activity of autochthonous bacteria isolated from Anabas testudineus (Bloch, 1792) and it's in vitro probiotic characterization.* Microb. Pathog., 113: 312–320, https://doi.org/10.1016/j.micpa th.2017.10.058, access: 19.05.2021.

MIDHUN S.J., NEETHU S., VYSAKH A. 2017b. *Antibacterial activity and probiotic characterization of autochthonous Paenibacillus polymyxa isolated from Anabas testudineus (Bloch, 1792).* Microb Pathog 113: 403–411, https ://doi.org/10.1016/j.micpath.2017.11.019, access: 19.05.2021.

MUKHERJEE A., GHOSH K. 2016. *Antagonism against fish pathogens by cellular components and verification of probiotic properties in autochthonous bacteria isolated from the gut of an Indian major carp, Catla catla (Hamilton).* Aquac. Res., 47: 2243–2255, https://doi.org/10.1111/are.12676, access: 19.05.2021.

NAZ S.A., RASOOL A.S. 2013. *Isolation, production and characterization of bacteriocins Produced by strains from indigenous Environments.* Pak. J. Bot., 45(1): 261–267.

NWABUEZE A.A., CAMPUS A., CAMPUS A. 2014. *Antimicrobial action of epidermal mucus extract of Clarias gariepinus (Burchell, 1822) juveniles-fed ginger inclusion in diet.* International Journal of Biology, 6(2): 42–8.

RAHMAN S., CHOUDHURY J.K., DUTTA A., KALITA M.C. 2014. *Eel Ichthyofauna of Assam in folklore therapeutic practices.* International Journal of Interdiscipline and Multidiscipline Studies, 1(5): 273–276.

RAY A.K., GHOSH K., RINGO E. 2012. *Enzyme-producing bacteria isolated from fish gut: a review.* Aquac. Nutr., 18: 465–492, https://doi.org/10.1111/j.1365-2095.2012.00943.x, access: 19.05.2021.

RITCHIE K.B. 2006. *Regulation of microbial populations by coral surface mucus and mucus-associated bacteria.* Mar. Ecol. Prog. Ser. 322, 1–14, doi: 10.3354/ meps322001, access: 19.05.2021.

SEBASTIAN J.M., SAHADEVAN N., VYSAKH A., RADHAKRISHNAN E.K., MATHEW J. 2018. *Antagonism against fish pathogens by cellular components/preparations of Bacillus coagulans (MTCC-9872) and It's in vitro probiotic characterisation.* Springer of Nature, https://doi.org/10.1007/s00284-018-1506-0, access: 19.05.2021.?

Seiler C., Berendonk T. 2012. *Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture*, Frontiers in Microbiology, 3: 399.

Sichewo P.R., Gono R.K., Muzondiwa J., Mungwadzi W. 2014. *Isolation and identification of pathogenic bacteria in edible fish: a case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe*, International Journal of Current Microbiology and Applied Sciences, 3(11): 897–904.

Soomro A.H., Musad T., Sammiand S., Rathore H.A. 2007. *Comparison of different methods for detection of antimicrobial activity of Lactobacillus* spp. Pakistan Journal of Zoology, 39(4): 265–268.

Subramanian S., Mackinnon S.L., Ross N.W. 2007. *A comparative study on innate immune parameters in the epidermal mucus of various fish species*. Comparative Biochemistry and Physiology, 148B: 256–263.

Tyor A.K., Kumari S. 2016. *Biochemical characterization and antibacterial properties of fish skin mucus of fresh water fish, Hypophthalmichthys nobilis*. International Journal of Pharmaceutical Science, 8(6): 6–10.

Vennila R., Kumar K.R., Kanchana S., Arumugam M., Vijayalakshmi S. 2011. *Preliminary investigation on antimicrobialand proteolytic property of the epidermal mucus secretion of marine stingrays*. Asian Pacific Journal of Tropical Biomedics, 1(2): S239–243.

Wei O.Y., Xavier R., Marimuthu K. 2010. *Screening of antibacterial activity of mucus extract of snakehead fish, Channa striatus (Bloch).* European Review of Medical and Pharmacology Science, 14: 675–681.

WHO. 2017. *Food safety and food-borne illness.* World Health Organization Fact sheet, Geneva.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 36(3): 333–358, Y. 2021

OVERVIEW ANALYSIS OF CULTURAL ECOSYSTEM SERVICES: MAPPING INDICATORS AND CATEGORIES CLASSIFICATION

***Asmaa Abualhagag*[1], *István Valánszki*[2]**

[1] ORCID: 0000-0002-1925-6140
[2] ORCID: 0000-0002-0779-6537
[1,2] Department of Landscape Protection and Reclamation, Institute of Landscape Architecture, Urban Planning and Garden Art
Hungarian University of Agriculture and Life Sciences, Budapest, Hungary
[1] Department of Architecture Engineering
Aswan University, Aswan, Egypt

Key words: cultural ecosystem services, mapping indicators, mapping methods, classification group, stated preference, revealed preference.

Abstract

Mapping of Cultural Ecosystem Services (CES) emphasizes the spatial contribution to landscape characteristics like land cover and human wellbeing. This review paper aims to build an overview of CES mapping indicators and methods. This goal comprises many objectives; to provide an overview of existing mapping indicators and methods, to analyze and classify them, and to emphasize important challenges that researchers face whilst mapping them. This study reviews 45 publications from the last ten years and identifies eight common CES mapping methods and various mapping indicators. In conclusion, we highlight that: 1) It is necessary to utilize a symmetric classification systems for each CES category and a clear specification of each category of CES. 2) there are various combinations of CES mapping indicators and methods. 3) it is important to combine different mapping methods, to map neglected services like education and culture heritage.

Introduction

Millennium Ecosystem Assessment (MEA) defines the concept of Ecosystem Services (ES) as a tool for sustainable development and mentions that ES consists of main four categories: cultural services (CES), regulating, supporting, and provisioning (LEE et al. 2019, MARTIN et al. 2016). Moreover, the first category of CES are defined as non-material ecosystem's benefits which contribute to human wellbeing like recreation, aes-

Address: Asmaa Abualhagag, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary, 1118, Budapest, Villányi út 29-43, e-mail: asmaa.haggag@aswu.edu.eg

thetic, and education services (Cheng et al. 2019). Although CES contribute to increase national economies by promoting recreation and tourism industry, recent research has indicated that researchers and policymakers pay limited attention to CES compared to the other categories of ES (Lee et al. 2019). CES mapping methods have functional and practical application (e.g. landscape design and urban planning) and support policymaking (Martin et al. 2016). In the context of culture services, 'Mapping' means the process of measuring, modeling, and quantifying the non-monetory and monetory value of something. Scientific literature has shown various specifications and expressions to recognize particular methodological approaches, the most common synonyms of mapping, including measuring, modeling, accounting, quantifying, valuation, etc. There are many different classifications of CES mapping methods. For example, The Economics of Ecosystems and Biodiversity (TEEB) mostly has classified these methods into preference-based and biophysical methods (TEEB. 2010). Moreover, Brown et al. (2017) and Lindholst et al. (2015) have classified preference-based mapping methods into the monetary method and non-monetary method.

Since CES are inherently invisible and nonmaterial services, the mapping of CES remains poorly understood and relatively ignored (Martin et al. 2016, Langemeyer et al. 2015). Furthermore, the limited availability of indicators and data connected with mapping culture services are a considerable challenge for quantifying all categories of ES, especially CES (Martin et al. 2016). According to MEA natural ecosystems are degrading, and CES is being utilized unsustainably and that result in increasing ecosystem degradation around the world. Because of the urgent need to protect CES, new policies have been established. Furthermore, the necessity of CES mapping indicators to measure and quantify them is growing around the world. Therefore, scientific research on CES has increased basically in the past decade (Hutcheson et al. 2018, Stanik et al. 2018).

Due to grows the interest in CES, the need to quantify and account for them is also growing through modeling and mapping. There are various benefits of mapping and modeling CES. For instance, the information from modeling can be used to estimate establish trends and costs and trade-offs (e.g. Sinclair et al. 2019, Paracchini et al. 2014). Thus, CES scientific research around the world must share one main goal: maintenance of practices and policies to ensure the sustainable provision of CES and related humans' wellbeing benefits. Unfortunately, most of CES cannot be mapping directly, therefore consider the utilize of mapping indicators necessary and classified them based on their natural for accounting and measuring of CES categories. Due to produce reliable and accurate results in

CES modeling, strong quantification is desired. Thus, it is necessary to indicate the proper indicators utilized for modelling and mapping CES as a first step in order to develop reliable and feasible indicators for modeling and mapping of each category of CES. In that regard, this review paper aims to collect, and overview analysis of previous studies concentrating on CES mapping indicators and classified them based on each CES category. These indicators will be utilized for mapping and quantifying various categories of CES and identify used data sources to enable visualize CES on maps by illustrating; 1) the most common CES mapping indicators and mapping methods; 2) the availability of data source and the extent of data. To achieve the research aims, we addressed the following research questions; 1) what indicators can be utilized in mapping CES categories? 2) what are the methods can be used for mapping different types of CES? 3) what indicators are used for all types of CES? And 4) what indicators are specific to one type of CES?

Materials and Methods

Paper selection

In this study a comprehensive search of ScienceDirect and of Scopus was conducted, using the search terms "mapping cultural ecosystem services", "quantifying methods of cultural ecosystem service", "valuation cultural ecosystem service" and "mapping indicators" in order to identify existing literature dealing specifically with mapping CES by applying this search keywords in main titles and abstracts. The literature review was not be limit by a fixed period or performed in a specific country. The search was perfumed from November 2020 to January 2021. The utilized search terms bring about a total of 220 publications including conference papers, journal articles, reports and thesis. After, in-depth screening of papers, we conducted 45 papers that have been read in-depth and considered in our analysis and comparison. From each analyzed paper, data about the CES mapping methods and indicators have been extracted, and extract general data about the used study area, such as data extent, data source, study area (for more details see App. 1, Table App. 1)

Analytical framework. Mapping indicators and methods classification

The analyzed literature review contained information about measuring CES by using appropriate indicators and methods. In case of mapping methods, in our paper selection we only focusing on paper utilized non-monetary methods for mapping CES categories, therefore, in this review, we discuss all kind of non-monetary mapping methods. According to non-monetary methods which classified to revealed preference and stated preference method, the revealed preference method means, analyzing documents or monitoring behavior including advertisements, pictures, and written data, to indirectly locate human's preference for CES (Sinclair et al. 2019). In contrast, the stated preference method means to, directly asking people about their preference to measuring CES (Wartmann and Purves 2018). Based on our analysis, there are different papers conduct and general review of publications addressed various categories of CES. For example, Figueroa-Alfaro and Tang (2017) reviewed 36 publications associated with CES mapping. Cooper et al. (2016) also performed a comparative review of 97 articles about the characteristics and availability of CES mapping methods. However, In this review, we:

a) update the list of CES mapping methods;

b) classify and group the CES mapping indicators;

c) indicate the utilized indicators in mapping each category of CES;

d) highlight the common utilized data source and extent in mapping various category of CES.

Mapping methods classification

We classified all considered publications based on their mapping method and only focusing on papers which used non-monetary methods. In this classification, we distinguished the mapping methods using revealed preference from the mapping methods using stated preference method (Schirpke et al. 2016, Riechers et al. 2018). Based on this classification, in this review paper, we intend to scan each paper to classify them according to used methods, and number of indicators used in mapping each CES category, then after the analysis of the total set of selected publications, we give a list of mapping indicators that could be used in mapping various category of CES.

Results

CES categories

According to our analysis, all CES categories have received some attention in the selected literature. Out of the 45 publications, 29 studies have addressed outdoor recreation and tourism category, so, they received the greatest attention among other categories, and only 11 studies mapped aesthetic enjoyment value. However, spiritual and inspirational value have received the least attention among other CES categories (3 and 2 studies mapping them respectively) – Table 1.

Table 1

Number of studies per each CES category

Culture services	Number of studies
Aesthetic enjoyment	11
Inspiration value	3
Recreation and tourism	29
Spiritual value	2

Classification groups of indicators

In this paper, we review existing indicators for the assessment of CES categories and provide a critical overview of how indicators can be used for mapping CES categories. Different indicators can be used to map different categories of CES. Based on indicators identified by gathered literature review, in this review, we divided the type of CES mapping indicators into four groups, namely active-physical interactions with the natural environment (Gr1), passive-physical interactions with the natural environment (Gr2), representative and intellectual interactions with the natural environment (Gr3), and spiritual, symbolic interactions with the natural environment (Gr4), based on Common International Classification of Culture Ecosystem Services (CICCES) (HAINES-YOUNG and POTSCHIN-YOUNG 2018)

The Classes/groups definitions indicating the different kinds of CES have all been addressed in Table 2. At the classification level of CES based on the characteristics of living systems, there are two kind of CES division which is between those characteristics of living systems that are experienced either 'in-situ' or 'remote'. For example, divide 1 is "Direct interactions with living systems that depend on presence in the environmental setting'. However, the second divide is 'Indirect interactions with living systems that do not require presence in the environmental setting' (HAINES-YOUNG et al. 2016).

Table 2
International Classification of culture ecosystem services (CICCES) (HAINES-YOUNG et al. 2016)

<table>
<tr><th>Division</th><th colspan="2">Group</th><th>Classes</th></tr>
<tr><td rowspan="5">Direct interactions with living systems</td><td>Gr_1</td><td>active-physical interactions with the natural environment</td><td>living systems characteristics that enable activities which promote public health and enjoyment through interactions</td></tr>
<tr><td>Gr_2</td><td>passive-physical interactions with the natural environment</td><td>living systems characteristics that enable activities promoting health and enjoyment through passive interactions</td></tr>
<tr><td rowspan="3">Gr_3</td><td rowspan="3">representative and Intellectual interactions with the natural environment</td><td>living systems characteristics that enable education and training</td></tr>
<tr><td>living systems characteristics that are resonant in terms of culture or heritage</td></tr>
<tr><td>Characteristics of living systems that enable aesthetic experiences,</td></tr>
<tr><td>Indirect interactions with living systems</td><td>Gr_4</td><td>spiritual, symbolic interactions with natural environment</td><td>elements of living systems that have sacred or religious meaning</td></tr>
</table>

Mapping indicators

In this section, we provide an overview of the mapping indicators used in the literature for mapping different categories of CES. The majority of mapping indicators have been used to map different CES categories (ABUALHAGAG and ISTVAN 2020). To be more specific, a various kind of indicators such as number of visitors, photographs, tourist attractions and landscape aesthetics, utilized for measuring recreation and tourism. Moreover, there are many indicators could be used for mapping different kind of CES categories such as land cover, and accessibility/ distance. Table 3 illustrate CES mapping indicators and the group of CES categories.

CES mapping indicators utilized as input data for mapping and evaluation CES category. Outdoor recreation and tourism had the greatest different number of mapping indicators account around 29 different kinds of indicators compared to all other CES categories (Table 3). Based on our result, land use and land cover indicators demonstrate to be a necessary indicator for mapping all CES categories. Land use indicator is commonly defined as a series of operations on land, carried out by humans. However, the land cover indicator is commonly defined as the vegetation (natural or planted) or man-made constructions (buildings, etc.) which occur on the earth surface. Land use and land cover have some fundamental differences. Land use refers to the purpose the land serves, for example, recreation, wildlife habitat, or agriculture; it does not describe the surface cover on the ground like a land cover indicator (TENERELLI et al. 2016). Vegetation types is an important map for mapping recreation and tourism while land use can be used for quantifying suitable and non-suitable areas for create new recreation services (UPTON et al. 2015, STANIK et al. 2018, TENERELLI et al. 2016).

In this paper, we review existing indicators for the assessment of CES categories and provide a critical overview of how indicators can be used for mapping CES categories. Different indicators can be used to map different categories of CES. Based on indicators identified by gathered literature review, this study identified four aspects to group the collected mapping indicators. These indicators comprise environmental aspects (including temperature, pollution, topography indicators such as DEM and slope, landscape settings); physical aspects (including the accessibility indicators such as distance indicator, which include distance to resources, distance to scenic site, flower viewing indicator, tourist attractions, population density, and roads); socio-economic aspects (including photographs, number of visitors, accommodation, footpaths, visitors stay, and visitors expenses); and urban aspects (including land cover and land use, resource availability, vegetation cover, rare species, green spaces, recreation potential, ecotourism potential, and protected areas – see Table 3 which illustrates the indicators for mapping CES resources).

Table 3

Classification groups of culture ecosystem services and related indicators

Division	Groups	Culture services (CES)	Number of studies	Mapping indicators aspects	CES mapping indicators	Number of studies
Direct interactions with living systems	passive-physical and experiential interactions with natural environment	aesthetic enjoyment value	11	environmental aspects	DEM	4 out of 11
				environmental aspects	slope	3 out of 11
				environmental aspects	temperature	1 out of 11
				physical aspects	distances	1 out of 11
				physical aspects	distance to resources	1 out of 11
				physical aspects	distance to scenic site	1 out of 11
				urban aspects	land use	1 out of 11
				urban aspects	green spaces	1 out of 11
				urban aspects	land cover	3 out of 11
				urban aspects	rare species	1 out of 11

cont. Table 3

Direct interactions with living systems	active-physical and experiential interactions with natural environment	recreation and tourism	29	socio-economic aspects	number of visitors	9 out of 29
				socio-economic aspects	photographs	3 out of 29
				socio-economic aspects	tourist attractions	2 out of 29
				environmental aspects	landscape aesthetics	3 out of 29
				urban aspects	recreation potential	1 out of 29
				urban aspects	ecotourism potential	1 out of 29
				environmental aspects	fresh water	2 out of 29
				urban aspects	recreation fishing	3 out of 29
				urban aspects	land cover	14 out of 29
				physical aspects	accessibility/ distance	7 out of 29
				physical aspects	traffic census	1 out of 29
				socio-economic aspects	footpaths	1 out of 29
				socio-economic aspects	population density	4 out of 29
				urban aspects	urban green space	1 out of 29
				socio-economic aspects	tourist attractions	2 out of 29
				environmental aspects	rare species	1 out of 29
				socio-economic aspects	accommodation	4 out of 29
				environmental aspects	resource availability	1 out of 29
				socio-economic aspects	flower viewing	1 out of 29
				socio-economic aspects	visitors expenses	1 out of 29
Indirect interactions with living systems	spiritual, symbolic and other interactions with natural environment	inspiration value	3	urban aspects	land cover	2 out of 3
				urban aspects	landscape value	1 out of 3
				urban aspects	land use	1 out of 3
Indirect interactions with living systems	spiritual, symbolic and other interactions with natural environment	spiritual value	2	socio-economic aspects	photographs	1 out of 2
				urban aspects	landscape settings	1 out of 2
				physical aspects	distance to resources	1 out of 2

The most common mapping indicators are the accessibility and the distance (e.g. distance from roads, distance to resources, distance from exist recreation site, and distance from water supply) and both are used for mapping nearly all CES categories. Table 3 highlights many examples of mapping indicators utilized for mapping different CES categories. Aesthetic enjoyment value has received more attention than other CES categories except recreation and tourism, and around a quarter of the selected studies addressed aesthetic enjoyment. Thus, our results show that 11 studies have mapped aesthetic enjoyment. So, aesthetic enjoyment has approximately 20 mapping indicators. However, it still less than indicators used for mapping outdoor recreation and tourism. Distance (include distance to resources and distance to Scenic site) was the important indicator utilized for mapping aesthetic enjoyment value. Land use and land cover data were also necessary information for measuring and quantified this service.

According to inspiration value, various kinds of indicators could be utilized for mapping this kind of service. Approximately, three indicators have been utilized to map inspiration category (Table 3). Recreation and tourism category received the most attention between other CES categories (approximately 64% of the analyzed studies mapped them) (Table 2). Thus, it is obvious that there are various kinds of mapping indicators related to the recreation and tourism category compared to other CES categories. For example, in case of recreation and tourism category, our results identified that there are 29 indicators have been utilized to quantifying and measuring them (Table 3) (van Berkel and Verburg 2014). Many studies approve that spiritual value is more difficult to measure and quantify. Therefore, it has been received the least attention among all CES categories. All mapping indicators utilized for quantifying this category were connected to the diversity of habitat. Moreover, fewer indicators utilized to map this service compared to other CES categories (Table 3). The significantly lower numbers of mapping indicators for spiritual value could be the cause of the limited data and indicators on these services. Because of these challenges, spiritual experience value received the least attention based on our analysis.

CES mapping methods

In this section, we give a general overview of CES mapping methods typically utilized to measure and map CES categories. By analysis the 45 collected studies included in this review we conclude that many different sets of non-monetary mapping methods, like revealed preference and stated preference methods, were found. The non-monetary mapping methods utilized to map CES categories received most attention in all the analyzed studies. Based on that, eight non-monetary mapping methods have been identified, of which the first 3 mapping methods utilized revealed preference methods for measuring and quantifying CES categories namely; observation, document, social media-based, and the rest utilized stated preference methods namely; interview, questionnaire, participatory mapping, participatory GIS (PGIS), public participation GIS (PPGIS), and scenario simulation. The descriptions of non-material methods for mapping CES as the following:

1) Revealed preference; this method consist of three main kinds:

a) observation: looks at user and locals' behavior and actions to reflect the social value of CES. For instance, remarking the number of visits to the park to evaluate the significance of recreation value in this area;

b) document: collecting information about human preferences on CES by looking for images, texts, or other kinds of materials. For instance, analyzes the kind and number of pictures taken by users to assess the aesthetic value;

c) social media-based: utilizing the data collected from various social media to evaluate CES. For example, analysis of the pictures of wildlife uploaded on a picture-sharing online website.

2) Stated preference: this method consist of five main kinds:

a) interview: directly understanding the perception of the public about why and how users are value CES by using face-to-face interviews. through this interview, participants talk freely about their thoughts and feelings to gain a better understanding of CES services like a sense of place;

b) questionnaire: a combination of questions is distributed to obtain information about CES from participantprze, for example, the planners ask users to choose from the set of selections;

c) participatory GIS (PGIS): in this method, the researcher integrates geographic information systems (GIS) and the participatory mapping method in the mapping process;

d) scenario simulation: predict future scenarios of CES capacities to help decision-makers and planners in the planning strategies

According to the non-monetary methods, overall, participatory GIS (PGIS) and observation were most frequently used for mapping CES categories far more than the others, followed by questionnaires, document and scenario simulation methods (Figure 1). Moreover, questionnaires and interviews frequently utilized observation and document methods to collect data for mapping CES. Figure 2 shows which of these methods are utilized to map different categories of CES. Overall, all mentioned mapping methods were utilized to map and quantify tourism and recreation categories, followed by aesthetic enjoyment value. In that regard, the above-mentioned mapping methods were utilized to measure and quantify different kinds of CES. Moreover, interviews, questionnaires, and participatory GIS methods have the capability to map all CES categories.

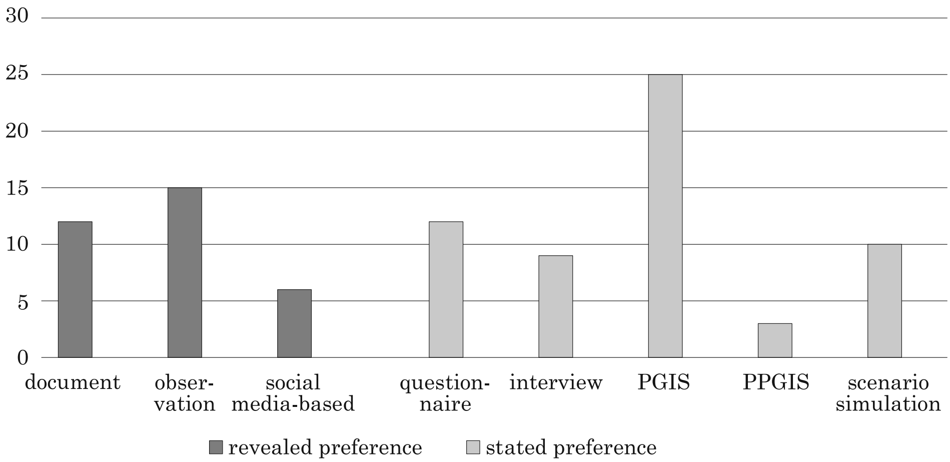


Fig. 1. Numbers of papers using various non-material methods to map culture ecosystem services

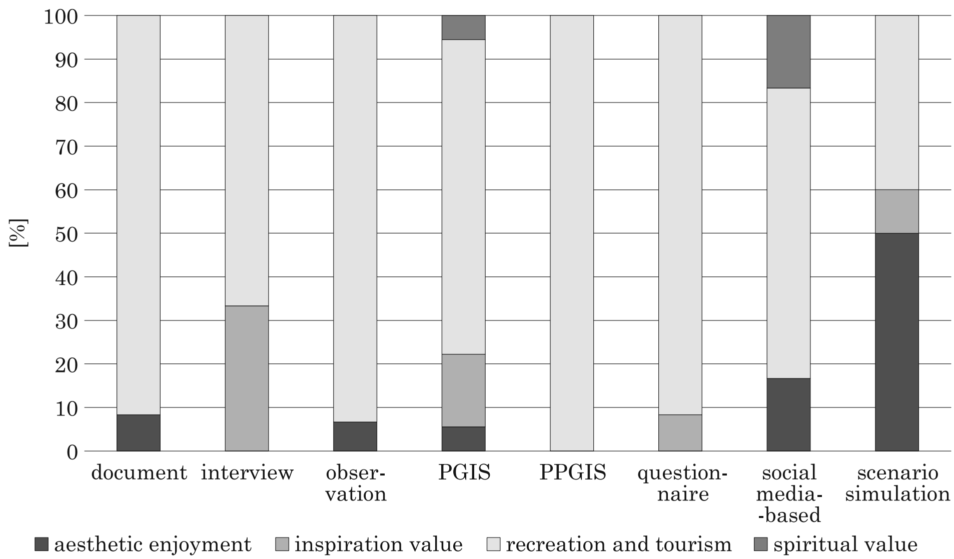


Fig. 2. CES various category per different mapping methods

Table 4 illustrate which CES category are mapping with revealed preference, and which of them are mapping with stated preference. Overall, recreation and tourism are used most frequently in case of revealed preference (29 out of 65) and stated preference methods (36 out of 65), followed by aesthetic enjoyment, in which stated preference methods are used more than revealed preference. Specifics of the quantification mapping methods' numbers per CES are given in App. 1, Table App. 1

Table 4

Number of mapping methods of CES per category

Culture services	Methods classifications		Total
	revealed preference	stated preference	
Aesthetic enjoyment	3	11	14
Inspiration value	0	8	8
Recreation and tourism	29	36	65
Spiritual value	1	1	2

Utilized Data Sources

The essential element in the mapping of the culture ecosystem categories is the availability of data sources and information. In that regard, this section gives an overview of kind information and data source utilized for measuring and mapping CES categories in the analyzed studies. So, we have focused on identifying the mapping indicators and analyzing them in order to indicate the type of data and information utilizing for mapping various categories of CES. Moreover, we first gathered the mapping indicators as these were utilized as a basis for mapping CES categories. After gathering the mapping indicators, the collected data source has been classified into three main groups namely; sub-national scale data, national scale data, and local data which is created for evaluation special study area (Table 5). These groups help the planners to identify the proper data type for each planning scale.

Table 5

Extent of data used in the reviewed literature, linked with the CES categories

Extent of data	CES categories				Total [%]
	aesthetic enjoyment [%]	recreation and tourism [%]	inspiration value [%]	spiritual value [%]	
Local scale	2	25	0	0	27
Sub-national scale	8	28	0	2	38
National scale	10	19	4	1	34

Overall, based on the analyzed literature review, many different types of data sources have been addressed, these data sources have been divided into three main types, as we mentioned before; sub-national data (e.g. specific case study, states, cities, regions, or watersheds), local data (e.g. monitoring and observations of study area features), and data covering national extents (e.g. countries). Of the 45 studies analyzed in this review, 38% mapped CES using sub-national data and 34% used national data and 27% used local data (Table 5). According to the sub-national data sources, there are a wide variety of data sources used to mapping a different kind of CES categories such as land use/cover map, pollution data, visitor numbers, etc. So, Cultural services, like aesthetic enjoyment and spiritual value, are very regional service with diversity from cultural groups to individuals; thus, sub-national data sources are mostly used for mapping and quantifying process.

The kind of data used in the analyzed studies had various nature and sources and utilized quantified method (see App. 1, Table App. 1). According to the most common data sources, there are various kind of sources used to map and evaluate various categories of CES, such as interviews and field data (FIGUEROA-ALFARO and TANG 2017), and other researchers used surveys which based on photographs or pictures. Furthermore, others utilized spatial source of data, such as maps about vegetation, land cover, or land use map. And the most and accurate source of data is written data and maps which can be directly collected from local public and private institutions. The number of studies used the various kind of data source have been reported in Table 6 linked with utilized extent of data and each CES categories. Overall, of the 45 studies included in this review (Table 6), 80% mapped CES using GIS and paper-based maps or GIS file maps such as using PPGIS method in case of paper-based map and using PGIS methods in case of GIS file maps. And around 24% used picture for mapping CES categories and only 40% used written data (see App. 1, Table App. 1 for more details).

Table 6

Type of data used in the reviewed literature, linked with the CES categories

Type of data	Utilized extent of data	Number of studies				Total (45)	Percentage
		aesthetic enjoyment [%]	recreation and tourism [%]	inspiration value [%]	spiritual value [%]		
Maps	local, national, sub-national	10	21	3	2	36	80
Pictures	sub-national, local	2	8	0	1	11	24
Written data	local	1	17	0	0	18	40

Discussion and Conclusions

Challenges of mapping methods application in ces categories

In this section, we give an overview about; firstly, discuss challenges of mapping CES; secondly, discuss the existing CES mapping indicators and the most common methods.This study identified various possible indicators and data sources for mapping each CES categories in practical applications. In the CES mapping process, all CES categories have the same necessity and the researchers should pay more attention to all CES (Ribeiro and Ribeiro 2016). In this study, our overview shows how the mentioned methods utilized for mapping CES concentrate mostly on measuring and mapping tourism and recreation values. Due to achieving a better addressing of all culture service in practical application, it is necessary to refer to the most common classification frameworks of CES categories which are MEA and TEEB frameworks. Thus, in many cases, the assessment of CES ends up as a symbolic evaluation of the CES concept, concentrating on demonstrating the utilization of CES classification framework without conceptual clarification like what kind of indicators should map or measure. The recreation category can easily be defined in various kind of classification systems, for example in in case of TEEB classification systems it called 'recreation and tourism', while it named 'recreation and ecotourism' in case of MEA classification systems classification systems and Common International Classification of Ecosystem Services (CICES), so it is important to refer to all CES to the most common classification frameworks.

Moreover, for other CES categories, it is hardly finding definitions or synonyms in the mentioned two international classification systems. Therefore, the comparison and analysis of these studies could be difficult, in the case of researchers utilize different classification systems. Due to these challenges, some scientific researchers addressed only a single clear CES category, such as Nahuelhual et al. (2013) addressed recreation value and Zarkesh et al. (2011) conducted a land evaluation of ecotourism value, at the same time all the other CES categories have been ignored. Based on the mentioned challenge, it appears that the practical application of CES mapping methods may still be problematic. CES are more than just 'recreation and tourism. Thus, the field of CES research needs to apply more rigorous definitions in the various case studies by single and clear classification systems and unambiguous descriptions of each CES category.

CES mapping indicators

Cultural ecosystem services (CES) are strongly connected with human well-being. However, up to now specific definitions and strong measurements of the necessity of cultural services for people have been elusive. A better understanding of this kind of service could offer feedback changes in ecosystem service, in general, and contribute to sustainable use and improvement. Our review paper indicates that there is an increase of literature addressed evaluating and mapping CES. Despite these advances, the sources of utilized data and information and mapping methods are varied, and in the majority of the analyzed studies, detailed methodological information and mapping indicators were missing.

Our review exposes some clear trends. Key CES that are today being utilized for decision-making in urban planning have been frequently mapped, as is the case of outdoor recreation (SINCLAIR et al. 2019) and tourism facilities (NAHUELHUAL et al. 2013). Yet, it is notable that CES that may be critical for the maintenance of human well-being, such as education and economic value, are rarely addressed. There is a clear lack of formal research on many of these kind of cultural services

Regarding the type of indicators aspects found in the review, indicators assessing urban aspects were the most frequently used for mapping aesthetic, inspiration, spiritual, and recreational services. For example, KOMOSSA et al. (2018) utilized accessibility and distance as urban indicator for mapping outdoor recreation. However, BEECO et al. (2014) conduct evaluation of recreation by using tracking number of visitors as a Socio-economic aspect's indicator.

Yet indicators measuring impacts on human well-being were only rarely addressed, although existing research connecting recreational activities and human well-being. In term of the CES quality assessment, it is evident that the reviewed cultural services indicators are generally lacking in terms of public participants of the subject to be measured, which may lead to confounding outcomes (SCHNEIDER and LORENCOVÁ 2015). For example, in case of Ives et al. (2017) and BIELING (2014), they Invested more effort for involving relevant stakeholders in the evaluation and mapping process, and that would likely improve their quality. Communication strategies to disseminate indicators were barely apparent in the literature, although the indicators assessed seemed to sufficiently reach their target audiences by using suitable communication means (FIGUEROA-ALFARO and TANG 2017).

Our results approve the fact that recreation and tourism services are mapped utilizing many various indicators (BERNETTI et al. 2019, BIELING

2014). Therefore, the number of indicators using in map recreation and tourism services significantly increased. In that regard. the possibility of using the obtained indicators for mapping all categories of CES are ambiguous and need more research to prove that. As we mentioned in the result section, there are different mapping indicators collected from the analyzed studies, and some of the obtained indicators can be suitable for one study area and non-suitable for others. Moreover, in this review, we confirm and referred to a list of CES mapping indicators with related groups and aspects.

CES Mapping Methods and data source

The stated preference methods, as a kind of CES mapping methods, have received more attention compared to other mapping method. Moreover, stated preference methods could be utilized to map many different categories of CES (Brown et al. 2016). Neglected CES services like education and cultural heritage can be mainly measured and modeled by the stated preference methods, like questionnaires, interviews, PGIS, and PPGIS, since these categories are depending mainly on user's perception (Clemente et al. 2019). Additionally, these mapping methods depend on the answers directly gathered by users with various socioeconomic and demographic backgrounds (D'amato et al. 2016, Ribeiro and Ribeiro 2016). Moreover, collection of the required information for revealed preference mapping methods is comparatively easy. They are often utilized in the cross-regional study area since there is no need to understand the local languages in this type of mapping. Accurate and precise measuring is the major challenge, due to people's personalities and perceptions of CES value. This result highlights that little is known about the accuracy of data collection, thus, improving the accuracy of quantification results and providing a well-designed measuring process controlled by a well-experienced researcher is required (Dou et al. 2017).

Mapping mainly refers to the process of quantifying the value of something. From the short review above, the results demonstrate two things. Firstly, we found that most of the analyzed studies utilized more than one mapping method for measuring a single category of CES. Secondly, many different methods were applied in a similar sequence in the mapping process. For example, for mapping CES categories, two main steps have to be followed; firstly, data evaluation has to be obtained by utilizing revealed preference methods, like observation and document methods; secondly, measuring CES categories has to be applied by using state preference methods, like questionnaires (e.g. Richards and Tunçer 2018), interviews (e.g. Riechers 2018), and PPGIS (e.g. Rall et al. 2019). Among them, various data source – like photo, maps, and written data- are often utilized repeatedly to verify and improve the research accuracy. Additionally, revealed preference mapping methods are frequently utilized to improve and verify data accuracy. For example, PARACCHINI et al. (2014) conducted interviews and workshops several times during the field survey and mapping process to better design the final questionnaire and to increase the accuracy of the obtained data. Additionally, these methods do not require a large sample set to collect accurate data, because the mapping process can be done by gathering in-depth perspectives from many people who live in a particular study area.

The kind of data utilized in the analyzed studies had different sources and nature, according to the used mapping method. As stated above, most of the selected publications utilized data acquired from personal interviews and field surveys. For example, USAMA (2015) conduct an interview with locals to discuss urban design guidelines in Cairo, Egypt that promote the physical activity of users and to motivate and change social behavior towards healthy living. The personal interview could be an online interview or face-to-face. Moreover, other surveys were based on various photographs or pictures. For example, RICHARDS and TUNÇER (2018) used a social media photographs for cultural ecosystem services assessment. Moreover, others utilized spatial data such as maps about vegetation cover and land use/land cover, and others collect mapping data from written sheets (e.g. WEYLAND and LATERRA 2014). In general, most of the obtained studies utilized a combination of data sources; for example, NESBITT et al. (2017) use picture sources as the data source and used maps to conduct the spatial analysis on the selected picture of the CES by using social media photographs as a measurement unit. Thus, few studies used one data source in the evaluation process, like, PENG et al. (2019) used written data as the primary data source to evaluate CES values by using a questionnaire. Therefore, we support interdisciplinary and transdisciplinary collaboration in the CES mapping process, with particular attention to the skills from social sciences and its' methods to better advance and support the assessment process techniques. Furthermore, more mapping methods and procedures must be developed to evaluate neglected CES like education and economic values which obtained from recreation and tourism services. Finally, more research is required on how to integrate the results of the mapping methods into the practice framework of reality.

To sum up, in this review the advantages of the combination of stated and revealed preference methods have been discussed through our previous analysis. Moreover, this review was given a general overview of common CES mapping/quantify indicators and methods and used data types.

We can conclude as the following; firstly, at the beginning of the mapping process, the researcher used, e.g., document, observation, expert-based methods to obtain information about CES and clarify and classify them. Secondly, questionnaires, interviews, participatory mapping, etc., are used to assess CES as the final step in the mapping process. This combination addressed a clear and accurate process for researchers to follow it. However, we must know the challenges of conducting this mapping process. Therefore, we emphasize many of challenges, which have been addressed above, facing researchers through the mapping process. The first challenge is that a well-experienced researcher is needed who is familiar with a various mapping methods and techniques, and the obtention of the proper data source and measuring method need a well-recognized researcher. Second, although questionnaires, interviews, participatory mapping, etc., are strongly encouraged to be utilized in the final step of mapping process, it is challenging to find accurate indicators and data sources of some particular service, consequently, it results in the inability to CES integration into the ES framework.

Accepted for print 29.10.2021

References

ABUALHAGAG A., VALÁNSZKI I. 2020. *An analytical framework of cultural ecosystem service mapping methods*. International conference of Scientific Meeting of Young Researches, pp. 8–23.

BACHI, L., RIBEIRO, S.C., HERMES, J., SAADI, A. 2020. *Cultural ecosystem services (CES) in landscapes with a tourist vocation. Mapping and modeling the physical landscape components that bring benefits to people in a mountain tourist destination in southeastern Brazil*. Tourism Management, 77: 104017.

BEECO J.A., HALLO J.C., BROWNLEE M.T. 2014. *GPS visitor tracking and recreation suitability mapping: Tools for understanding and managing visitor use*. Landscape and Urban Planning, 127: 136–145

BERNETTI I., CHIRICI G., SACCHELLI S. 2019. *Big data and evaluation of cultural ecosystem services: an analysis based on geotagged photographs from social media in tuscan forest (Italy).* Forest-Biogeosciences and Forestry, 12(1): 98–110.

BIELING C. 2014. *Cultural ecosystem services as revealed through short stories from residents of the Swabian Alb (Germany).* Ecosystem Services, 8: 207–215.

BRENNER J., JIMENEZ J.A., SARDA R., GAROLA A. 2010. *An assessment of the non-market value of the ecosystem services provided by the Catalan coastal zone, Spain*. Ocean & Coastal Management, 53(1): 27–38.

BROWN G., PULLAR D., HAUSNER V.H. 2016. *An empirical evaluation of spatial value transfer methods for identifying cultural ecosystem services*. Ecological indicators, 69: 1–11.

BROWN G., STRICKLAND-MUNRO J., KOBRYN H., MOORE S.A. 2017. *Mixed methods participatory GIS. An evaluation of the validity of qualitative and quantitative mapping methods*. Applied Geography, 79: 153–166

CHAN, K.M.A., SHAW, M.R., CAMERON, D.R., UNDERWOOD, E.C., DAILY, G.C. 2006. *Conservation planning for ecosystem services*. PLoS biology, 4(11): e379.

CHENG X., VAN DAMME S., LI L., UYTTENHOVE P. 2019. *Evaluation of cultural ecosystem services. A review of methods*. Ecosystem Services, 37: 925–100.

CLEMENTE P., CALVACHE M., ANTUNES P., SANTOS R., CERDEIRA J.O., MARTINS M.J. 2019. *Combining social media photographs and species distribution models to map cultural ecosystem services. The case of a Natural Park in Portugal*. Ecological Indicators, 96: 59–68.

COOPER N., BRADY E., STEEN H., BRYCE R. 2016. *Aesthetic and spiritual values of ecosystems. recognising the ontological and axiological plurality of cultural ecosystem 'services'*. Ecosystem Services, 21: 218–229.

D'AMATO D., REKOLA M., LI N., TOPPINEN A. 2016. *Monetary valuation of forest ecosystem services in China. A literature review and identification of future research needs*. Ecological Economics, 121: 75–84.

DOU Y., ZHEN L., DE GROOT R., DU B., YU X. 2017. *Assessing the importance of cultural ecosystem services in urban areas of Beijing municipality*. Ecosystem Services, 24: 79–90.

EIGENBROD F., ARMSWORTH P.R. ANDERSON B.J., HEINEMEYER A., GILLINGS S., ROY D.B., THOMAS C.D., GASTON K.J. 2010. *The impact of proxy-based methods on mapping the distribution of ecosystem services*. Journal of Applied Ecology, 47(2): 377–385.

FIGUEROA-ALFARO R.W., TANG Z. 2017. *Evaluating the aesthetic value of cultural ecosystem services by mapping geo-tagged photographs from social media data on Panoramio and Flickr*. Journal of Environmental Planning and Management, 60(2): 266–281.

GASCOIGNE W.R., HOAG D., KOONTZ L., TANGEN B.A., SHAFFER T.L., GLEASON R.A. 2011. *Valuing ecosystem and economic services across land-use scenarios in the Prairie Pothole Region of the Dakotas, USA*. Ecological Economics, 70(10): 1715–1725.

GOSAL A.S., ZIV G. 2020. *Landscape aesthetics. Spatial modelling and mapping using social media images and machine learning*. Ecological Indicators, 117: 106638.

GRÊT-REGAMEY A., WALZ A., BEBI P. 2008. *Valuing ecosystem services for sustainable landscape planning in Alpine* regions. Mountain Research and Development, 28(2): 156–165.

HAINES-YOUNG R., POTSCHIN-YOUNG M. 2018. *Revision of the common international classification for ecosystem services (CICES V5. 1): a policy brief*. One Ecosystem, 3: e27108.

HAINES-YOUNG R., POTSCHIN-YOUNG M., CZÚCZ B. 2016. *Report on the use of CICES to identify and characterise the biophysical, social and monetary dimensions of ES assessments*. Deliverable D4, 2.

HEIN L., VAN KOPPEN K., DE GROOT R.S., VAN IERLAND E.C. 2006. *Spatial scales, stakeholders and the valuation of ecosystem services*. Ecological Economics, 57(2): 209–228.

HELIAN L., SHILONG W., GUANGLEI J., LING Z. 2011. *Changes in land use and ecosystem service values in Jinan, China*. Energy Procedia, 5: 1109–1115.

HUANG S.L., CHEN Y.H., KUO F.Y., WANG S.H. 2011. *Emergy-based evaluation of peri-urban ecosystem services*. Ecological Complexity, 8(1): 38–50.

HUTCHESON W., HOAGLAND P., JIN D. 2018. *Valuing environmental education as a cultural ecosystem service at Hudson River Park*. Ecosystem Services, 31: 387–394.

IVES C.D., OKE C., HEHIR A., GORDON A., WANG Y., BEKESSY S.A. 2017. *Capturing residents' values for urban green space. Mapping, analysis and guidance for practice*. Landscape and Urban Planning, 161: 32–43.

KENWARD R.E., WHITTINGHAM M.J., ARAMPATZIS S., MANOS B.D., HAHN T., TERRY A., SIMONCINI R., ALCORN J., BASTIAN O., DONLAN M., ELOWE K. 2011. *Identifying governance strategies that effectively support ecosystem services, resource sustainability, and biodiversity*. Proceedings of the National Academy of Sciences, 108(13): 5308–5312.

KOMOSSA F., VAN DER ZANDEN E.H., SCHULP C.J., VERBURG P.H. 2018. *Mapping landscape potential for outdoor recreation using different archetypical recreation user groups in the European Union*. Ecological Indicators, 85: 105–116.

LANGE G.M., JIDDAWI N. 2009. *Economic value of marine ecosystem services in Zanzibar. Implications for marine conservation and sustainable development*. Ocean & Coastal Management, 52(10): 521–532.

LANGEMEYER J., BARÓ F., ROEBELING P., GÓMEZ-BAGGETHUN E. 2015. *Contrasting values of cultural ecosystem services in urban areas. The case of park Montjuïc in Barcelona*. Ecosystem Services, 12: 178–186.

Lara A., Little C., Urrutia R., McPhee J., Álvarez-Garretón C., Oyarzún C., Soto D., Donoso P., Nahuelhual L., Pino M., Arismendi I. 2009. *Assessment of ecosystem services as an opportunity for the conservation and management of native forests in Chile*. Forest Ecology and Management, 258(4): 415–424.

Lautenbach S., Kugel C., Lausch A., Seppelt R. 2011. *Analysis of historic changes in regional ecosystem service provisioning using land use data*. Ecological indicators, 11(2): 676–687.

Lee H., Seo B., Koellner T., Lautenbach S. 2019. *Mapping cultural ecosystem services 2.0–potential and shortcomings from unlabeled crowd sourced images*. Ecological indicators, 96: 505–515.

Limaei S.M., Safari G., Merceh G.M. 2017. *Non-market valuation of forest park using travel cost method (case study: Saravan forest park, north of Iran)*. Austrian Journal of Forest Science, 134(1): 53–74.

Lindholst A.C., Caspersen O.H., Van den Bosch C.C.K. 2015. *Methods for mapping recreational and social values in urban green spaces in the nordic countries and their comparative merits for urban planning*. Journal of Outdoor Recreation and Tourism, 12: 71–81.

Martin C.L., Momtaz S., Gaston T., Moltschaniwskyj N.A. 2016. *A systematic quantitative review of coastal and marine cultural ecosystem services: current status and future research*. Marine Policy, 74: 25–32.

Mea M.E.A. 2005. *Ecosystems and human well-being: wetlands and water synthesis*.

Müller F., de Groot R., Willemen L. 2010. *Ecosystem services at the landscape scale: the need for integrative approaches*. Landscape Online, 23: 1–11.

Nahuelhual L., Carmona A., Lozada P., Jaramillo A., Aguayo M. 2013. *Mapping recreation and ecotourism as a cultural ecosystem service: An application at the local level in Southern Chile*. Applied Geography, 40: 71–82.

Naidoo R., Weaver L.C., De Longcamp M., Du Plessis P. 2011. *Namibia's community-based natural resource management programme: an unrecognized payment for ecosystem services scheme*. Environmental Conservation, 38(4): 445–453.

Nesbitt L., Hotte N., Barron S., Cowan J., Sheppard S.R. 2017. *The social and economic value of cultural ecosystem services provided by urban forests in North America. A review and suggestions for future research*. Urban Forestry & Urban Greening, 25: 103–111.

O'Farrell P.J., De Lange W.J., Le Maitre D.C., Reyers B., Blignaut J.N., Milton S.J., Atkinson D., Egoh B., Maherry A., Colvin C., Cowling R.M. 2011. *The possibilities and pitfalls presented by a pragmatic approach to ecosystem service valuation in an arid biodiversity hotspot*. Journal of Arid Environments, 75(6): 612–623.

Paracchini M.L., Zulian G., Kopperoinen L., Maes J., Schägner J.P., Termansen M., Bidoglio G. 2014. *Mapping cultural ecosystem services. A framework to assess the potential for outdoor recreation across the EU*. Ecological Indicators, 45: 371–385.

Peng W., Liu W., Cai W., Wang X., Huang Z., Wu C. 2019. *Evaluation of ecosystem cultural services of urban protected areas based on public participation gis (ppgis). A case study of Gongqing Forest Park in Shanghai, China, ying yong sheng tai xue bao*. Journal of Applied Ecology, 30(2): 439–448.

Posthumus H., Rouquette J.R., Morris J., Gowing D.J.G., Hess T.M. 2010. *A framework for the assessment of ecosystem goods and services; a case study on lowland floodplains in England*. Ecological Economics, 69(7): 1510–1523.

Rall E., Hansen R., Pauleit S. 2019. *The added value of public participation GIS (PPGIS) for urban green infrastructure planning*. Urban Forestry & Urban Greening, 40: 264–274.

Raudsepp-Hearne C., Peterson G.D., Bennett E.M. 2010. *Ecosystem service bundles for analyzing tradeoffs in DIVERSE landscapes*. Proceedings of the National Academy of Sciences, 107(11): 5242–5247.

Reyers B., O'Farrell P.J., Cowling R.M., Egoh B.N., Le Maitre D.C., Vlok J.H. 2009. *Ecosystem services, land-cover change, and stakeholders: finding a sustainable foothold for a semiarid biodiversity hotspot*. Ecology and Society, 14(1), doi: 10.5751/ES-02867-140138.

Ribeiro F.P., Ribeiro K.T. 2016. *Participative mapping of cultural ecosystem services in Pedra Branca State Park, Brazil. Natureza & Conservação*, 14(2): 120–127.

RICHARDS D.R., TUNÇER B., 2018. *Using image recognition to automate assessment of cultural ecosystem services from social media photographs*. Ecosystem Services, 31: 318–325.

RIECHERS M., BARKMANN J., TSCHARNTKE T. 2018. *Diverging perceptions by social groups on cultural ecosystem services provided by urban green*. Landscape and Urban Planning, 175: 161–168.

SANDHU H.S., WRATTEN S.D., CULLEN R., CASE B. 2008. *The future of farming. The value of ecosystem services in conventional and organic arable land. An experimental approach*. Ecological Economics, 64(4): 835–848.

SCHIRPKE U., TIMMERMANN F., TAPPEINER U., TASSER E. 2016. *Cultural ecosystem services of mountain regions. Modelling the aesthetic value*. Ecological Indicators, 69: 78–90.

SCHNEIDER J., LORENCOVÁ H. 2015. *Recreational activities, practices and attitudes of visitors to the protected landscape areas as a basis for resolving conflicts of recreation and nature protection*. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 63(5): 1555–1564.

SHERROUSE B.C., CLEMENT J.M., SEMMENS D.J. 2011. *A GIS application for assessing, mapping, and quantifying the social values of ecosystem services*. Applied Geography, 31(2): 748–760.

SHI L., CUI S. 2009. *Impacts of land use/cover change on ecosystem services for Xiamen*. In AGU Fall Meeting Abstracts, 2009: B33B–0399.

SINCLAIR M., GHERMANDI A., MOSES S.A., JOSEPH S. 2019. *Recreation and environmental quality of tropical wetlands: A social media based spatial analysis*. Tourism Management, 71: 179–186.

STANIK N., AALDERS I., MILLER D. 2018. *Towards an indicator-based assessment of cultural heritage as a cultural ecosystem service – A case study of Scottish landscapes*. Ecological Indicators. Elsevier, 95: 288–297.

TEEB 2010. *The Economics of ecosystems and biodiversity: Ecological and Economic Foundations. In:* KUMAR P. (coordinating lead author: de Groot, R.), Earthscan, London & Washington.

TROY A., WILSON M.A. 2006. *Mapping ecosystem services: practical challenges and opportunities in linking GIS and value transfer*. Ecological Economics, 60(2): 435–449.

UPTON V., RYAN M., O'DONOGHUE C., DHUBHAIN A.N. 2015. *Combining conventional and volunteered geographic information to identify and model forest recreational resources*. Applied Geography, 60: 69–76.

USAMA A.N. 2015. *Urban space design to enhance physical activities and motivate healthy social behavior In Cairo, Egypt*. Proceedings Of Intcess 15- 2nd International Conference on Education and Social Sciences.

VAN BERKEL D.B., VERBURG P.H. 2014. *Spatial quantification and valuation of cultural ecosystem services in an agricultural landscape*. Ecological Indicators. Elsevier Ltd, 37: 163–174.

VAN JAARSVELD A.S., BIGGS R., SCHOLES R.J., BOHENSKY E., REYERS B., LYNAM T., MUSVOTO C., FABRICIUS C. 2005. *Measuring conditions and trends in ecosystem services at multiple scales. The Southern African millennium ecosystem assessment experience*. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1454): 425–441.

VIHERVAARA P., KUMPULA T., TANSKANEN A., BURKHARD B. 2010. *Ecosystem services A tool for sustainable management of human – environment systems. Case study Finnish forest lapland*. Ecological Complexity, 7(3): 410–420.

WARTMANN F.M., PURVES R.S. 2018. *Investigating sense of place as a cultural ecosystem service in different landscapes through the lens of language*. Landscape and Urban Planning. Elsevier, 175: 169–183.

WEYLAND F., LATERRA P. 2014. *Recreation potential assessment at large spatial scales. A method based in the ecosystem services approach and landscape metrics*. Ecological Indicators, 39: 34–43.

WILLEMEN L., HEIN L., VERBURG P.H. 2010. *Evaluating the impact of regional development policies on future landscape services*. Ecological Economics, 69(11): 2244–2254.

ZARKESH M.M.K., ALMASI N., TAGHIZADEH F. 2011. *Ecotourism land capability evaluation using spatial multi criteria evaluation*. Research Journal of Applied Sciences, Engineering and Technology, 3(7): 693–700.

Appendix 1

Table App. 1

Summary of studies and mapping indicators and method of the literature review

	Ecosystem service	Primary indicator (P)		Secondary indicators (S)		Extent of data	Type of data source	Used method non-monetary)	Extent of study area	Reference
1	aesthetic enjoyment (A)	PA_1	landscape aesthetics	SA_1	DEM	national	maps	scenario simulation	national	(GOSAL et al. 2020)
				SA_2	slope	national			national	
				SA_3	site	sub-national			local	
2	aesthetic enjoyment (A)	PA_2	aesthetics	SA_1	DEM	national	pictures	social media-based	local	(CLEMENTE et al. 2019)
3	aesthetic enjoyment (A)	PA_3	spiritual, aesthetic,	SA_2	slope	national	written data	observation	local	(BIELING 2014)
				SA_4	distance	sub-national	maps	PGIS,	local	
				SA_5	green spaces	sub-national	maps	PGIS	local	
4	aesthetic enjoyment (A)		aesthetic value	SA_3	site	local data	maps	scenario simulation	local	(SHERROUSE et al. 2011)
				SA_1	DEM	national			national	
				SA_2	slope	sub-national			national	
5	aesthetic enjoyment (A)	PA_4	nature appreciation	SA_6	rare species	local data	pictures, maps	document	local	(RAUDSEPP-HEARNE et al. 2010)
6	aesthetic enjoyment (A)	PA_1	aesthetic landscape	SA_7	land cover	national	maps	scenario simulation	local	(VIHERVAARA et al. 2010)
7	aesthetic enjoyment (A)	PA_5	nature reserves basin	SA_8	distance to resources	sub-national	maps	PGIS	local	(SHI AND CUI 2009)
		PA_6	national scenic spot	SA_9	distance to scenic site	sub-national	maps	PGIS	local	
8	aesthetic enjoyment (A)	PA_7	scenic beauty	SA_1	DEM	national	maps	scenario simulation	local	(GRÊT-REGAMEY et al. 2008)
				SA_7	land cover	national			local	
				SA_{10}	temperature	sub-national			local	
9	aesthetic enjoyment (A)	PA_2	aesthetics	SA_{11}	land use	national	maps	PGIS	national	(SANDHU et al. 2008)
10	aesthetic enjoyment (A)	PA_8	aesthetic and amenities	SA_7	land cover	national	maps	PGIS	local	(TROY et al. 2006)
11	aesthetic enjoyment (A)	PA_3	spiritual, aesthetic, recreational services	SA_{12}	protected areas	sub-national	maps	scenario simulation	local	(VAN JAARSVELD et al. 2005)
12	recreation and tourism (R)	PR1	scenic beauty, heritage, leisure	SR_1	number of visitors	*local data*	written data	observation,	local	(BACHI et al. 2020)

				SR_2	photographs	local data	pictures	social media-based,	local	
				SR_3	tourist attractions	local data	maps	participatory gis (pgis),	local	
13	recreation and tourism (R)	PR2	aesthetic and recreation	SR_4	landscape aesthetics	national	maps,	participatory gis (pgis), observation	local	(RALL et al. 2019)
				SR_1	number of visitors	local data	written data		local	
14	recreation and tourism (R)	PR2	recreation and aesthetic	SR_2	photographs	local data	pictures	social media-based, interview,	local	(WARTMANN and PURVES. 2018)
				SR_1	number of visitors	local data	written data	interview, observation	local	
15	recreation and tourism (R)	PR3	recreational	SR_5	recreation potential	national	maps	scenario simulation	local	(LIMAEI et al. 2017)
				SR_6	ecotourism potential	national	maps		local	
16	recreation and tourism (R)	PR4	recreational and ecotourism	SR_3	tourist attractions	local data	maps , pictures,	observation , interview, participatory gis (pgis)	national	(TENERELLI et al. 2016)
				SR_7	rare species	local data	maps, pictures,		national	
				SR_8	tax value of accommodation	local data	written data		national	
				SR_9	forested cover	national	maps		national	
17	recreation and tourism (R)	PR3	recreation	SR_{10}	fresh water	national	maps,	participatory gis (pgis), questionnaire	national	(COOPER et al. 2016)
				SR_{11}	recreation fishing	local data	written data, pictures			
18	recreation and tourism (R)	PR5	forest recreation	SR_{12}	accessibility	sub-national	maps	participatory gis (pgis),	national	(VAN BERKEL and VERBURG 2014)
				SR_{13}	land cover	national	maps		national	
				SR_{14}	distance	sub-national	maps		national	
19	recreation and tourism (R)	PR3	recreation	SR_{15}	urban green space	sub-national	maps	questionnaire	local	(KENWARD et al. 2011)
20	recreation and tourism (R)	PR6	tourism	SR_{16}	flower viewing	local data	pictures, written data	social media-based, observation	local	(O'FARRELL et al. 2011)
				SR_{17}	viewsheds	local data			local	
				SR_1	visitors numbers	local data			local	
21	recreation and tourism (R)	PR3	recreation	SR_{18}	water fowls	sub-national	maps	participatory GIS (PGIS)	local	(GASCOIGNE et al. 2011)
22	recreation and tourism (R)	PR7	land cover	SR_9	forest cover	national	maps	PPGIS	local	(HUANG et al. 2011)
23	recreation and tourism (R)	PR3	recreation	SR_{13}	land cover	national	maps	scenario simulation, PGIS	local	(HELIAN et al. 2011)

24	recreation and tourism (R)	PR8	outdoor recreation	SR_{13}	land cover	national	maps	PPGIS, questionnaire	local	(LAUTENBACH et al. 2011)
				SR_{19}	traffic census	local data	maps	observation	local	
25	recreation and tourism (R)	PR6	ecotourism	SR_{20}	resource availability	national	maps	document	national	(NAIDOO et al. 2011)
		PR9	trophy hunting	SR_{20}	resource availability	national	maps		national	
26	recreation and tourism (R)	PR2	aesthetics and recreation	SR_{13}	land cover	national	maps	scenario simulation	local	(BRENNER et al. 2010)
27	recreation and tourism (R)	PR3	recreational use	SR_{1}	visitors numbers	local data	written data	observation	national	(EIGENBROD et al. 2010)
28	recreation and tourism (R)	PR10	potential recreational use	SR_{21}	footpaths	sub-national	maps	participatory GIS (PGIS), questionnaire, document	local	(POSTHUMUS et al. 2010)
				SR_{22}	cultural heritage	sub-national	written data		local	
				SR_{22}	distance to resources	sub-national	maps		local	
				SR_{23}	population density	sub-national	written data		local	
29	recreation and tourism (R)	PR11	number of tourist attractions	SR_{1}	visitors numbers	local data	written data	document, interview, observation	local	(RAUDSEPP-HEARNE et al. 2010)
		PR5	forest recreation	SR_{9}	forest cover	national	maps	participatory GIS (PGIS), questionnaire, document	local	
30	recreation and tourism (R)	PR3	recreation	SR_{13}	land cover	national	maps	participatory GIS (PGIS), questionnaire, document	local	(VIHERVAARA et al. 2010)
31	recreation and tourism (R)	PR12	accommodatio n suitability	SR_{13}	land cover	national	maps	participatory GIS (PGIS), questionnaire, document	local	(WILLEMEN et al. 2010)
				SR_{14}	distance	sub-national	maps		local	
					distance to resources	sub-national	maps		local	
				SR_{12}	accessibility	sub-national	maps		local	
					accommodation	local data	written data		local	
32	recreation and tourism (R)	PR3	recreational use	SR_{1}	visitor numbers	local data	written data	document, interview, observation	local	(MÜLLER et al. 2010)
33	recreation and tourism (R)	PR13	potential leisure cycling population	SR_{22}	distance to resources	sub-national	maps	participatory GIS (PGIS), questionnaire, document	local	(WILLEMEN et al. 2010)
				SR_{24}	roads	sub-national	maps		local	
				SR_{23}	population density	sub-national	written data		local	

		PR12	accommodation suitability	SR_{13}	land cover	national	maps		local	
				SR_{14}	distance	sub-national	maps		local	
				SR_{22}	distance to resources	sub-national	maps		local	
				SR_{12}	accessibility	sub-national	maps		local	
				SR_{25}	accommodation	local data	written data		local	
34	recreation and tourism (R)	PR14	potential tourism	SR_{17}	viewsheds	local data	pictures	questionnaire, observation	local	(Reyers et al. 2009)
35	recreation and tourism (R)	PR15	recreational fishing opportunities	SR_{26}	fish abundance	sub-national	pictures, written data,	observation	local	(Lara et al. 2009)
36	recreation and tourism (R)	PR16	international tourism	SR_{1}	visitors numbers	local data	written data, pictures	observation	national	(Lange et al. 2009)
				SR_{27}	visitors stay	local data		interview	national	
				SR_{28}	visitors expenses	local data		interview	national	
		PR17	fishing	SR_{26}	fish abundance	sub-national		social media-based	national	
				SR_{29}	fish consumption	sub-national			national	
37	recreation and tourism (R)	PR12	accommodation suitability	SR_{13}	land cover	national	maps	scenario simulation questionnaire, participatory GIS (PGIS)	local	(Willemen et al. 2010)
				SR_{14}	distance	sub-national	maps		local	
				SR_{22}	distance to resources	sub-national	maps		local	
				SR_{12}	accessibility	sub-national	maps		local	
				SR_{25}	accommodation	local data	written data		local	
		PR13	potential leisure cycling population	SR_{22}	distance to resources	sub-national	maps	observation, questionnaire	local	
				SR_{24}	roads	sub-national	maps	questionnaire	local	
				SR_{23}	population density	sub-national	written data	document, interview	local	
38	recreation and tourism (R)	PR3	recreation	SR_{15}	natural areas	national	maps	questionnaire	local	(Chan et al. 2006)
				SR_{12}	accessibility	sub-national	maps	participatory GIS (PGIS),	local	
				SR_{23}	population density	sub-national	maps, written data	document	local	
39	recreation and tourism (R)	PR3	recreation	SR_{1}	visitors numbers	local data	written data	document, observation	local	(Hein et al. 2006)

40	recreation and tourism (R)	PR3	recreation	SR_{13}	land cover	national	maps	public participation GIS (PPGIS),	local	(Troy et al. 2006)
41	inspiration value (I)	PI_1	cultural and spiritual	SI_1	land cover	national	maps	interview, participatory GIS (PGIS)	local	(Brenner et al. 2010)
42	inspiration value (I)	PI_2	landscape value	SI_2	landscape value	national	maps	interview, participatory GIS (PGIS)	local	(Posthumus et al. 2010)
				SI_3	land use		maps		local	
43	inspiration value (I)	PI_3	authenticity landscape	SI_3	land use	national	maps	interview, participatory GIS (PGIS)	local	(Willemen et al. 2010)
				SI_1	land cover	national	maps	scenario simulation, questionnaire	local	
44	spiritual value (S)	PS1	education value	SS_1	photographs	sub-national	pictures	social media-based,	local	(Langemeyer et al. 2015)
				SS_2	landscape settings	national	maps	participatory GIS (PGIS)	local	
45	spiritual value (S)	PS2	research and educational bases	SS_3	distance to resources	sub-national	maps	participatory GIS (PGIS)	local	(Shi and Cui 2009)