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ASSESSMENT THE EFFECT OF SEX ON SELECTED MORPHOMETRIC MEASUREMENTS AND SLAUGHTER YIELDS OF AFRICAN CATFISH *(CLARIAS GARIEPINUS* BURCHELL, 1822)

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Key words: African catfish, gender of fish, morphometric measurements, slaughter yield.

Abstract

This study was aimed at analyzing the results of morphometric measurements and slaughter yield of the carcass of African catfish depending on gender. The experimental material included 60 fish, with a gender ratio of 1:1 (females ♂: males ♀), cultured in the intensive system (pond culture). All fish were fed manually (every 3 h) with pelleted feed prepared at the farm. Morphometric measurements (body weight, total length, body length, side length of the head, head height, the smallest and the largest height of the body and body width) were performed in live fish, whereas *post-mortem* determinations were carried out for contents of particular elements of the carcass (head, viscera, fins, skin, bones and fillets). Results achieved demonstrated that male fish were characterized by greater body length, side length of the head and body height compared to females. They were also characterized by higher body weight, the yield of carcass and fillets without skin, and by higher contents of head, fins and bones in the total body weight. The statistical analysis showed the effect of sex on morphometric measurements and the selected parts of the body of catfish.

Introduction

African catfish (*Clarias gariepinus)* is one of the most important fish species currently being cultured both within and outside its natural range of tropical and subtropical environments (CHEPKIRUI-BOIT et al. 2011). It is one of the most commonly cultured, indigenous species of fish in Africa. It has an almost Pan African distribution (absent from Maghreb, the upper and lower Guinea, the Cape province, probably Nogal province), and also naturally occurs in Minor Asia: Jordan, Israel, Lebanon, Syria and south-

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ern Turkey (TEUGELS 1996). This species has been introduced in Europe, America and south-east Asia for aquaculture purposes (AGNESE et al. 1997). *C. gariepinus* is omnivorous, ability to eat a variety of natural (plant material, plankton, arthropods, mollusks, other fish, reptiles and amphibians) and accepts supplemental feeds, and is resistant to diseases, as well as this fish, can tolerate low oxygen and pH levels (MUSTAPHA et al. 2012). *C. gariepinus* has pseudo-lungs, long bodies and a high capacity to produce mucous as adaptations to live in stagnant environments or out of water (YALÇIN et al. 2001a, VITULE et al. 2006, AMISAH et al. 2009). The reproduction of this catfish is seasonal and linked with the maturation of gonads and it depends on water level, temperature and photoperiod. In the pond culture, females reach sexual maturity after 6–7 months, whereas the gonads of males become well-developed after 1.5–2.0 years (YALÇIN et al. 2001b, ADAMEK 2011). This catfish is sexually dimorphic, males have distinct urogenital sexual papillae, located behind the anus, which are absent in females (BARNHOORN et al. 2004).

Catfish and its hybrids are important worldwide. The total production of African catfish officially reported by FAO is 246,476 t during 2015 (FAO 2017); however, it is expected that the production will increase in the following years. Therefore, detailed characteristics of catfish and its hybrids' meat are of great importance to aquaculture and fish processing sectors in countries where catfish culture is very popular, such as Nigeria, Netherlands, Brazil, and Hungary. This approach is also in line with the Sustainable Development Goals (SDGs) set in 2015 by the United Nations which indicate the need of sustainable food production (UN 2015). In Europe, it was introduced in 1974 first in Cyprus and later in the Czech Republic, Slovakia and the Netherlands (GAVRILOAIE and CHIŞAMERA 2005). *C. gariepinus* is not only a very important aquaculture species in Poland but it is also included in the checklist of non-native fishes that occur in the fresh waters of Poland (NOWAK et al. 2008). According to ADAMEK (2011), fish with an average unitary body weight above 1200 g is the most desired in the Polish market. The intensive culture of catfish tends to achieve possibly the highest body weight of fish in a short period, which is feasible only under optimal conditions. Commercial culture enables the production of fish with a unitary body weight of 800–1000 g within 6–8 months. African catfish is a suitable alternative to tilapia, the yields of catfish from ponds could be as much as 2.5 times higher than those of tilapia (GODA et al. 2007). A high yield of both carcass and fillet are an additional advantage of this fish species. The average slaughter yield of *C. gariepinus* is 38.9%, and the content of total protein in meat reaches 18.6%. The use of industrial feed mixtures may contribute to an increased content of fat not only in meat but also in the whole body, thereby reducing carcass yield (JANKOWSKA et al. 2007, PUCHAŁA and PILARCZYK 2007, ADAMEK 2011). The meat of this species has an intense red color and a low natural loss, is tender and devoid of intense fishy flavor. In addition, the fillets are almost boneless. These factors make the meat of African catfish highly suitable for culinary and processing (SOBCZAK et al. 2022).

In Poland, *C. gariepinus* has been introduced in the early 90. However, the literature on its taxonomic status is still very scarce and the morphological characteristics of the species from the Polish aquaculture are limited (FILIPIAK et al. 1993, WIĘCASZEK et al. 2010). The morphometric characteristics of this species are significant, because (especially in Asian aquaculture) the hybrids of *C. gariepinus* are cultured with other species. Moreover, the closely related species like *C. anguillaris* or *C. macrocephalus* are cultured at a large scale, thus the detailed characteristics of the morphometric features may be needed to distinguish the different species and hybrids within the *Clarias* genus (WIĘCASZEK et al. 2010).

The available literature provides relatively little data on the technological evaluation of *C. gariepinus* carcasses originating from pond culture on differences to their sex. The research carried out can help farms specializing in the rearing of catfish to adjust the most optimum fattening period and sex choice to obtain the highest fish weight, as well as carcass and fillet yields. For this reason, a study was undertaken to see the effect of different sex (male/female) on the morphometric measurements and slaughter yield/fillet yield of African catfish.

Materials and Methods

Experimental fish, diets and origin

The experimental material included 60 fish of African catfish (*Clarias gariepinus*) with a body weight of approximately 1 kg and an age of 8 months, with a sex ratio of 1:1 (females ♂: males ♀). The fish were purchased after being caught during the autumn-winter season of 2016 at the Agricultural Farm specializing in the culture of fresh-water fish, located in the northern part of Poland. During rearing, the catfish were cultured in a concrete pond (in the intensive system) with a volume of 9,000 L and a closed circuit of water having a temperature of 25 ±1°C. The fish were fed manually (every 3 h) with pelleted feed prepared at the farm. The composition of feed was as follows (per 100 kg): 17.8 kg of fish meal, 44.6 kg of extracted soybean meal, 14.9 kg of wheat grain, 7.4 kg of corn grain, 11.9 kg of rapeseed cake, 2.4 L of fish oil, and 1 kg of a vitamin-mineral premix. Contents of basic nutrients in feed were determined at the Laboratory of the Department of Animal Nutrition and Fodder Science, University of Warmia and Mazury in Olsztyn (Poland), according to standard methods (AOAC 2005). The pelleted feed mixture contained: 33.57% of total protein, 5.82% of crude fat, 6.45% of crude ash, and 3.80% of crude fiber, whereas its energy value reached 17.229 MJ/kg.

Morphometric measurements and slaughter yield

The fish were caught 48 hours before slaughter and transferred to a separate pond at the farm and subjected to physiological cleansing, then stunned and slaughtered accordingly to standard procedures (EC 2009). The following measurements of live fish were taken (Figure 1): body weight, total length by using a ruler, body length and side length of the head, whereas with the use of a measuring caliper: head height, the smallest and the largest height of the body and body width. The selected morphometric measurements were taken according to WIĘCASZEK et al. (2010). Catfish pre-treatment included: manual evisceration (opening of body cavity, removal of viscera and blood clots), decapitation (cut behind epicranium outgrowths), removal of fins (cutting off: caudal, dorsal, abdominal and pectoral fins ca. 0.5 cm from the base) and filleting. Afterwards, the weight of particular body parts (head, viscera, fins, skin, bones and fillet) was noted using an electronic scale by Radwag (Radom, Poland) with the accuracy of 0.001 g.

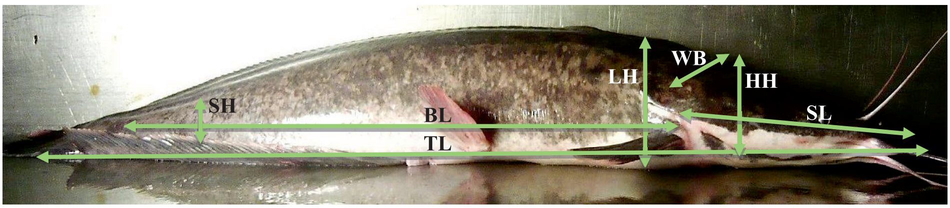

Fig. 1. Diagram of measurement points of morphometric evaluation of African catfish (HOLČIK 1989, WIĘCASZEK et al. 2010; with own modifications): TL – total length; BL – body length; SL – side length of the head; HH – the height of the head; LH – the largest height of the body; SH – smallest height of the body; WB – width of the body

Source: photo by I. Chwastowska-Siwiecka

Statistical analysis

The results were processed statistically by one-way analysis of variance in the Statistica computer software version 13.3 program (2017). They were presented in tables as mean values, standard deviation and standard error of the mean (SEM). The significance of differences ($P \leq 0.05$ and $P \leq 0.01$) between the mean values of the analyzed parameters was determined with the t-student's test.

Results and Discussion

The analysis of results of morphometric measurements of the evaluated fish species (Table 1) demonstrated that the total length and body length (without head and caudal fin) of males were significantly greater ($P \leq 0.01$) than females, by 3.67 and 2.51 cm, respectively. The male fish were also characterized by a greater side length of the head (by 1.14 cm), which was confirmed statistically ($P \leq 0.05$). In contrast, head height was similar in both sexes and reached 5.31 cm on average. Data collated in Table 1 demonstrate that the height measured (before caudal fin) at the smallest height of the body in males reached 4.09 cm and was statistically higher ($P \leq 0.01$) than in female fish. Simultaneously, in the group of ♂, was observed for higher values of the largest body height (7.06 cm). This experiment showed no effect of sex on the body width of African catfish. The statistical analysis of values of this morphometric parameter did not show any significant differences in the analyzed experimental groups, however, the females were characterized by greater body width, 7.49 vs. 6.72 cm, which could be due to the fact that they had already reached sexual maturity.

Table 1

Morphometric measurements of *C. gariepinus* (mean ± SD)

Specification	African catfish		SEM
	male (n = 30)	female (n = 30)	
Total length (TL) [cm]	54.55^{A} ±1.62	50.88^{B} ±1.43	0.537
Body length (BL) [cm]	47.33^{A} ±0.95	44.82^{B} ±0.46	0.331
Side length of the head (SL) [cm]	12.45^{a} ±0.80	11.31^{b} ±1.14	0.251
The height of the head (HH) [cm]	5.37 ±0.83	5.25 ±0.82	0.181
The largest height of body (LH) [cm]	7.06 ±0.79	6.56 ±0.36	0.145
The smallest height of body (SH) [cm]	4.09^{A} ±0.32	3.54^{B} ±0.39	0.100
Width of the body (WB) [cm]	6.72 ±0.59	7.49 ±1.00	0.200

Mean values denoted by different letters in the row are statistically significantly different at: $^{A, B}$ – $P \leq 0.01$; $^{a, b}$ – $P \leq 0.05$; SEM – standard error of means

The body sizes of fish play a significant role in the analysis of their utility value. From the technological perspective, less valuable within a species are small fish that are characterized by greater losses of body parts during manual pre-treatment of carcasses and lower fat content, as well as higher water holding capacity of meat. Fishes with a higher body weight allow obtaining regular pieces of meat, suitable for culinary and processing purposes (SKAŁECKI et al. 2008, KUŹMIŃSKI 2012, SKAŁECKI et al. 2013b). According to KLASA and TRZEBIATOWSKI (1992), African catfish – in the weight category of 951 to 1051 g – reached an average total length of 49.77 cm and body length of 45.6 cm. In a study by STANCHEVA et al. (2014), the total body length of European catfish with an average body weight of 3050 g was 65 cm, whereas in carp with a body weight of 1220 g it reached ca. 55 cm. In turn, as reported by SKAŁECKI et al. (2008), in cod with the average body weight of 1550 g the value of this parameter reached 59.25 cm and thus significantly exceeded the protective/commercial dimensions, which is set at 38 cm for this species. Investigations conducted by TURAN et al. (2005) point to great differences in morphological parameters in the *C. gariepinus* population inhabiting rivers of Turkey, which might be due to diversified environmental conditions, like temperature, turbidity, feed availability and depth of water. According to SOBCZAK et al. (2022), the sex of the fish (*C. gariepinus* x *Heterobranchus longifilis*) did not affect any of the tested biometric traits, although females had higher body (1169 g) and fillet weights (301,8 g), respectively. However, males had higher carcass weight (732 g) and carcass yield (64.4%).

Slaughter yield and meat quality of fish are determined by species, size, physiological condition, sex, age, motility of fish, environmental conditions (pH and temperature of water) as well as by the method, type of feeding and catch season (BUCHTOVA et al. 2007, MENOYO et al. 2007, GULER et al. 2008). An important parameter of utility value is also the content of edible parts in the carcass (SKAŁECKI et al. 2013a).

Based on results concerning the content of selected body parts of African catfish (Table 2), it can be stated that the mean weight of male fish reached 1062.80 g and was statistically higher ($P \leq 0.01$) compared to females by 109.20 g. In addition, the males were characterized by a significantly higher yield of the carcass without a head which accounted for 65.10%. Data presented in Table 2 and Figure 2 demonstrate that in the case of *C. gariepinus* females, the fillets constituted 41.82% of the total body weight, whereas in the case of males – 44.69% ($P \leq 0.01$). The male fish of the analyzed species were characterized by significantly higher head weight compared to females (by 1.93%), which was also confirmed in their higher body weight. The statistical analysis demonstrated a highly significant difference ($P \leq 0.01$) in the content of viscera which in the case of females was as high as 14.30%, whereas, in the case of males, it reached 6.51%.

Table 2

Percentage share of body parts of the carcass of *C. gariepinus* (mean ±SD)

Specification	African catfish		SEM
	male (n = 30)	female (n = 30)	
Body weight [g]	1062.80^{A} ±65.04	953.60^{B} ±49.93	17.699
Carcass without head [%]	65.10^{A} ±1.25	60.51^{B} ±1.85	0.628
Fillets [%]	44.69 ^{A} ±1.07	41.82 ^{B} ±1.22	0.413
Head [%]	27.2^{A} ±1.51	25.28^{B} ±1.76	0.421
Guts [%]	6.51^{B} ±0.34	14.30^{A} ±0.58	0.900
Fins [%]	5.06^{A} ±0.74	4.10^{B} ±0.89	0.209
Bones [%]	9.62^{A} ±0.82	7.51^{B} ±0.84	0.302
Skin [%]	5.85^{B} ±0.76	6.53^{A} ±0.67	0.173

Mean values denoted by different letters in the row are statistically significantly different at: $^{A, B}$ – $P \leq 0.01$; $^{a, b}$ – $P \leq 0.05$; SEM – standard error of means

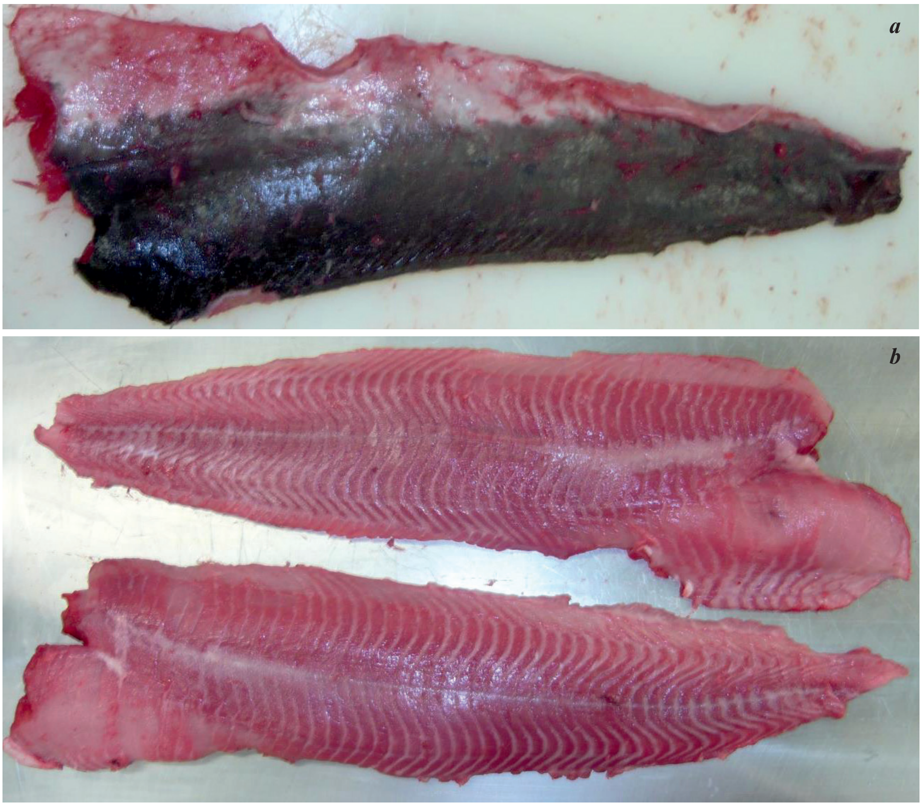

Fig. 2. Fillet of *C. gariepinus* with skin (*a*) and without skin (*b*)

Source: photo by I. Chwastowska-Siwiecka

This relatively large difference resulted from the presence of intensively developed female gonads filled with a high amount of roe, and viscera, constituting the majority of their body weight. Simultaneously, it was demonstrated that the content of bones separated during manual treatment of African catfish (♀) was lower (by 2.11%) compared to individuals from the other analyzed experimental group. In males, the average weight of fins reached 5.06% and was significantly higher than the percentage value determined for female individuals. In turn, an opposite statistical correlation was noted for the weight of skin, which was significantly higher in the females (6.53%) (Table 2). The research showed the share of non-edible by-products obtained from the carcass of females was 57.72% and was higher compared to males by 3.5% (Figure 3).

Fig. 3. Non-edible by-products of the carcass of *C. gariepinus*
Source: photo by I. Chwastowska-Siwiecka

African catfish is characterized by a relatively high technological yield of meat in both forms: carcass – 66.5%, fillet with skin – 51.6%, and fillet without skin – 45.4%. The total slaughter yield of catfish is satisfactory at mechanical processing and reaches 42 –43%, whereas at manual processing it accounts for 50–52%; in addition, it is correlated mainly with the size of fish (KLASA and TRZEBIATOWSKI 1992, KAPELIŃSKI 2003). The catfish is usually sold in fillets (ALFARO et al. 2014). Data reported by KLASA and TRZEBIATOWSKI (1992) concerning the effect of the sex of African catfish on the technological yield of the obtained elements indicate that during the processing of males it was higher by 3–4% than in females, however, the highest values were reported in the weight category of 1001–1051 g (ca. 5%). In the case of fillets with skin and skinned fillets, in the weight category of 951–1001 g, a slightly higher yield was noted in the female than male catfish (respectively: fillet with skin – by 2.8%, fillet without skin –

by 2.5%). According to SOUZA and MARENGONI (1998), the yield of catfish fillets averages 32.83%, while of by – products (for example: bones, skin and heads) obtained during processing account for about 67%. Catfish skin, comprising about 5% of the whole fish, has become an interesting raw material for gelatin production (ALFARO et al. 2014). The study by SKAŁECKI et al. (2013a) on the weight of edible parts of rainbow trouts (aged 1+) and carps (aged 3+), demonstrated that in the case of the first fish species the contents of meat and fillets reached 43.80% and 54.30%, and were significantly higher (by respectively 13.17% and 16.64%) compared to the other fish species. The analysis of data achieved by these authors demonstrates that the carp were characterized by a significantly higher content of head (26.30%) and bones (14.37%), whereas the contents of skin and fins were similar in both species. According to MARCU et al. (2010), an increase in slaughter yield of carps was strongly correlated with their body weight, where in the case of fish with a body weight of 785 g it reached 50.68%, and in the case of fish with the body weight of 2010 g it reached up to 60.28%. When analyzing the percentage content of the main body parts of catfish, BUD et al. (2008) showed a higher slaughter yield (69.35%) and a higher share of meat (53.00%) to the compared to own study. Whereas in the case of the non-edible parts of the catfish, the cited authors reported a lower share of the head (20.80%) and fins (2.25%) in related to the total weight of those fish, than noted in own study.

Conclusions

Results of the study demonstrated that the morphometric characteristics such as total length, and body length were significantly higher in males. Simultaneously, they were characterized by a significantly higher value of the side length of the head and body height measured at the smallest height of the body. In comparing catfish of both sexes at age 8 months, it was concluded that the females were characterized by lower body weight, the yield of carcass and fillets without skin and a lower percentage share of head, fins and bones in the carcass.

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PRODUCTIVITY, EGG QUALITY, AND EGG COMPOSITION OF QUAIL SUPPLEMENTED WITH CASSAVA LEAF PASTE

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Key words: cassava leaf paste, egg whites, egg yolk, performance, thick shell.

Abstract

Cassava leaf paste contains macro, micro, and bioactive mineral nutrients that improve physiological performance. The study aimed to evaluate the effect of adding cassava leaf paste on the productivity and quality of quail eggs. The parameters measured were performance, egg quality, and quail egg composition. The results showed that the application of cassava leaf paste increased quail body weight gain. The average quail eggshell that received cassava leaf paste was thicker than the control group. The percentage of quail egg whites and egg yolks that received cassava leaf paste treatment was higher than the control. This study concluded that quail treated with cassava leaf paste resulted in higher quail performance such as body weight gain and egg mass. Giving cassava leaf paste causes the quail to be healthier by producing a higher egg composition such as the percentage of egg whites and yolks and thicker eggshells.

Introduction

Quail has potential as an excellent and affordable source of animal protein, both for carcass and eggs produced (Marareni and Mnisi 2020). The Directorate General of PKH (2019) in 2018–2019 stated that the consumption of quail eggs in Indonesia per capita increased annually, by 7769

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to 9177 quail eggs, with a percentage increase of 0.18%. The consumption of quail eggs is followed by an increase in the quail population in Indonesia. The quail population in Indonesia from 2018 to 2019 was 14 062 000 to 14 107 000, with a percentage increase of 0.32% (Ditjen PKH 2019). Quail also produce eggs with high productivity. Data from the Directorate General of PKH (2019) for 2018–2019 shows that the number of quail egg production in Indonesia increased, from 28 957 000 eggs to 29 090 000 eggs, with a percentage increase of 0.45%. Quail egg production in Indonesia in 2018–2019 increased by 0.46% (Rahmasari et al. 2022). Mone et al. (2016) stated that the temperature of the rearing environment is a factor that affects quail productivity.

An increase in quail egg production must be followed by an increase in egg quality (Kusumorini et al. 2021). Egg quality is an indicator to determine whether an egg is good (Vlaicu and Panaite 2022). Assessment of egg quality is very important to know so that people know the eggs that are consumed, namely eggs that have good quality (SASS et al. 2020). Kalvandi et al. (2022) stated that the factor that affects the quality of quail eggs is the temperature of the rearing environment.

Rehman et al. (2022) stated that the thickness of the shell and the Haugh Unit are indicators that describe the indicators of egg quality. A good shell thickness indicates that the thicker the shell size will help minimize the inside of the egg that is contaminated with the environment so that the contents of the egg still have good resistance (Kalvandi et al. 2022). Meanwhile, a thin egg shell will cause the eggs to easily crack or break (Zotte et al. 2019). Kalvandi et al. (2022) stated that the thickness of quail shells aged 65–121 days reared at 22°C was 0.17 mm, while those reared at 34°C for 8 hours per day were 0.16 mm. Quail aged 42 days given Moringa leaf powder got 0.17 mm of shell thickness compared to those that did not get 0.18 mm of Moringa leaf powder (Minj et al. 2022).

In addition, egg white quality, especially high egg white, is also an important measure in keeping eggs fresh (Krisnaningsih et al. 2022). The egg white height will affect the resulting Haugh Unit (Nasrushin et al. 2021). The higher the Haugh Unit value, the better the egg quality (Jumadin et al. 2022). A Haugh Unit score over 72 is categorized as AA quality eggs, 60–72 as A quality eggs, 31–60 as B quality eggs, and Haugh Unit value less than 31 are categorized as C quality eggs (Usda 2020).

One of the factors that affect the productivity and quality of quail eggs is the temperature of the rearing environment (Mone et al. 2016). Wasti et al. (2020) stated that the thermoneutral zone of poultry, including quail, is 18–21°C. High environmental temperatures that exceed the range of the quail comfort zone have an impact on decreasing the productivity and quality of quail eggs (MEHAISEN et al. 2019). Quail experiencing heat stress will reduce heat production by limiting feed intake, resulting in a reduced availability of nutrients needed by quail (KALVANDI et al. 2019). Quail will also release its heat by panting when the ambient temperature is above the comfort zone (WASTI et al. 2020). Quails at the critical time, there is a decrease in the availability of carbon dioxide (CO_2) in the quail's blood. The reduction of CO_2 causes a decrease in bicarbonate concentration. Meanwhile, bicarbonate is a material needed in the formation of egg shells (EL-TARABANY 2016).

The provision of cassava leaf paste is expected to increase the productivity and quality of eggs in quail. Cassava leaf paste contains high protein and low crude fiber (JUMADIN et al. 2022a). Therefore, this study was conducted to evaluate the effect of adding cassava leaf paste on productivity, egg quality, and egg composition of quail reared under natural conditions in the tropics, such as in Indonesia.

Material and Method

Place and animal ethics

This research was carried out in several places, namely in the Jaja Quail Animal Cage, Pilot Plant Seafast Center Laboratory of LPPM IPB, Physiology Laboratory, SKHB, and IPB. The procedures used in this study were by the rules of the Animal Ethics Commission of the School of Veterinary Medicine and Biomedical IPB (No. 007/KEH/SKE/V/2021).

Preparation of cassava leaf paste (*Manihot esculenta* Crantz)

Fresh cassava leaves were obtained in Rancabungur Village, Ranca Bungur District, Bogor Regency. The cassava leaves used are whole and undamaged leaves. The leaf part is the sixth leaf from the shoot. The leaves were first washed with clean water, then dried at room temperature. Then cut into small pieces to facilitate the process of crushing with a blender, then extracted. A total of ±50 grams of cassava leaf pieces are crushed in a blender using 125 mL of 70% ethanol for 3 minutes, intermittently every 1 minute. The solution of cassava leaves in ethanol is then filtered by a fine cloth, then the filtrate obtained is filtered again with a Buchner funnel using filter paper. The residue is washed with 75 mL 70% ethanol, then filtered again with a Buechner funnel. The filtrate is taken as a cassava leaf extract. Furthermore, the extract of the cassava leaves is evaporated for one hour at a temperature of 70°C, resulting in cassava leaf paste. The calculation of the dose of cassava leaf paste in this study was a dose conversion of quail with a body weight of 168 g given 5.29 mg/head/day (JUMADIN et al. 2017). Doses 2 and 3 are multiples of dose 1.

Preparation and maintenance of experimental animals

Experimental animals used were female quail of layer period (aged 42 days) as many as 160 tails. The cage used was a 4-storey colony cage with 16 plots, measuring 100 cm long x 30 cm wide x 20 cm high. Each plot was filled with 10 birds and their placement was done randomly. Each plot is equipped with excreta storage, lighting, feed and drinking containers. All cage plots were placed in open cages. Open cage equipped with thermostat digital.

Experimental design

The study used a completely randomized design. The treatment was giving cassava leaf paste to quail, consisting of 4 levels/dose of cassava leaf paste, namely P0 (0 mg/g), P_1 (5.29 mg/g), P2 (10.58 mg/g), and P3 (15.87 mg/g). The experiment was repeated 4 times. Each replication consisted of 10 tails. Giving cassava leaf paste to quail is given through drinking water. Give it in the morning. At 6 o'clock all quail were fed, for 1 hour. At the time of feeding, all drinking water is taken, so the quail are thirsty. After that, drinking water mixed with cassava leaf paste was given at each dose of 5.29 mg/g; 10.58 mg/g; and 15.87 mg/g. After the drinking water that has been mixed with cassava leaf paste is used up, then proceed with ordinary drinking water.

Research procedure

A total of 160 quails from the age of 42 days were fed commercial feed (which contains 2900 kcal/kg metabolizable energy, 22% protein, 7% fat, 7% crude fiber, 14% ash, 2.5–3.5% calcium, 0.6–1.0% phosphorus, 0.9% lysine, 0.4% methionine) (JUMADIN et al. 2022a). Feed and drinking water were provided ad libitum. Temperatures was recorded every morning, afternoon, evening and night at 06.00 WIB, 12.00 WIB, 18.00 WIB and 24.00 WIB. The provision of cassava leaf paste was carried out through drinking water in 100 mL at each treatment level.

The study was conducted by rearing quail for 30 days. Observation of egg production performance was carried out from the first day to the end of the study. Analysis of egg quality and egg composition was carried out at the end of the study.

Research variables

The variables observed in this study included performance, egg quality, and egg composition in quail. Observation of quail performance was detected by calculating body weight gain, egg mass, mortality, and morbidity. Performances such as egg mass are counted daily. Other performances, such as body weight gain, were weighed when the quails were 42 and 72 days old and mortality was calculated at the end of the study. Morbidity was observed every day.

Observation of quail performance during the production period was carried out on quail aged 42–72 days. Body weight gain was obtained based on the difference between final body weight (72 days old) and initial body weight (42 days old). Egg mass was obtained based on the total egg weight during the study. Mortality was obtained by dividing the number of quails that died divided by the number of quails kept multiplied by 100%. Morbidity was calculated by dividing the number of sick quail by the number of quail population during the study multiplied by 100% (MOLINA-LO´PEZ et al. 2017).

Observations on the quality of quail eggs included counting the thickness of the shell, the height of the egg white, and the height of the yolk. Egg quality analysis was carried out at the end of treatment (72 days old). Observation of the quality of quail eggs in the production period was carried out on quail aged 42–72 days. The number of shell thickness was obtained from the average results of measurements of the pointed, middle, and blunt part of the eggshell using a digital caliper. Analysis of egg white height and yolk height was measured using a digital caliper.

Observation of egg composition was detected by calculating the percentage of egg shell, percentage of egg white, and percentage of egg yolk. Analysis of egg composition was carried out at the end of treatment (72 days old). Percentage of shells was obtained based on the ratio of shell weight to egg weight multiplied by 100%. Egg white percentage was calculated by dividing the egg white weight by the egg weight multiplied by 100%. Yolk percentage was obtained based on the ratio of yolk weight to egg weight multiplied by 100% (ŞENGUL and ÇALISLAR 2020).

Data analysis

This study was designed using a completely randomized design, with the treatment of giving cassava leaf paste to quail in the production period consisting of 4 levels/dose, namely P0 (0 mg), P1 (5.29 mg), P2 (10.58 mg), and P2 (10.58 mg). P3 (15.87 mg). The study was repeated 4 times. Each treatment, each replication consisted of 10 quails.

Data on quail productivity, egg quality, and egg composition were analyzed for variance using a mathematical model as follows:

$$Yij = \mu + Pi + \varepsilon ij,$$

where:
Yij – the observation value of the *i* cassava leaf paste and the *j* replication
μ – general mean
Pi – the effect of the *i* cassava leaf paste application
εij – treatment error of the *i* cassava leaf paste treatment and the *j* replication.

If real results are obtained, it is continued with Duncan's test (MATJIK and SUMERTAJAYA 2013).

Results and Discussion

Quail performance

Observations of quail performance in this study included body weight gain, egg mass, mortality, and morbidity. The results of these observations are presented in Table 1.

Table 1

Performance on quail given cassava leaf paste for 30 days

Variable	P0	P1	P2	P3
PBB [g/head/month]	6.5 ±0.49[b]	7.6 ±3.49[b]	13.43 ±3.95[a]	14.30 ±5.15[a]
Egg mass [kg]	2.92 ±0.26	2.93 ±0.36	3.21 ±0.17	3.03 ±0.31
Mortality [%]	0	0	0	0
Morbidity [%]	0	0	0	0

Numbers accompanied by different letters in the same row indicate significantly different ($p < 0.05$); P0 – cassava leaf paste 0 mg/g; P1 – cassava leaf paste 5.29 mg/g; P2 – cassava leaf paste 10.58 mg/g; P3 – cassava leaf paste 15.87 mg/g; PBB – weight gain

Different levels of cassava leaf paste in this study resulted in significant differences in body weight gain (PBB) of quail. The results showed that there was an increase in body weight (PBB) of quail with increasing doses of cassava leaf paste. This is because the quail is still in the early stages of production. MONE et al. (2016) stated that quail energy needs at the beginning of production are not only used to produce eggs but also for quail growth, especially the increase in their reproductive organs.

The quail egg mass showed no significant difference at various levels of cassava leaf paste administration. The value of egg mass that received cassava leaf paste was higher than the control. ASHOUR et al. (2020) stated that the nutritional content and mineral elements are factors that support the mass of quail eggs. Another factor that influenced the difference in the mass of quail eggs in this study was egg production and egg weight between treatments. NEMATI et al. (2021) stated that quail egg mass was influenced by egg production and quail egg weight.

Quail treated with cassava leaf paste produced the same mortality and morbidity rates as the control treatment. These results illustrate that the increase in the level of cassava leaf paste in this treatment was able to maintain quail in a healthy condition with a mortality rate of 0%.

Egg quality

Observation of the quality of quail eggs in this study was carried out by measuring the thickness of the shell, the height of the egg white, and the height of the yolk. The results of these observations are presented in Table 2.

Table 2

Quality of quail eggs given cassava leaf paste for 30 days

Variable	P0	P1	P2	P3
Shell thickness [mm]	0.15 ±0.05^{b}	0.20 ±0.00^{a}	0.20 ±0.00^{a}	0.20 ±0.00^{a}
Egg white height [mm]	4.50 ±0.94	4.63 ±0.42	5.03 ±0.35	4.40 ±0.57
Yolk height [mm]	11.82 ±0.81	11.70 ±0.71	12.35 ±0.25	11.57 ±0.51

Numbers accompanied by different letters in the same row indicate significantly different ($p < 0.05$); P0 – cassava leaf paste 0 mg/g; P1 – cassava leaf paste 5.29 mg/g; P2 – cassava leaf paste 10.58 mg/g; P3 – cassava leaf paste 15.87 mg/g

Statistically, the average shell of quail eggs that received cassava leaf paste was thicker than the group of quail eggs that did not get cassava leaf paste. JUMADIN et al. (2022) stated that flavonoids were able to reduce oxidative stress so that the deposition of calcium as the main element in the formation of egg shells from the small intestine into the egg cell was more efficient. The advantage of producing a thick egg shell is that it is not easy to crack or break. Thus, the chance of exposure to microbes is smaller (WIDYANTARA et al. 2017).

Quail that received cassava leaf paste produced higher egg white values than those in the control treatment, except for P3. JUMADIN et al. (2022a) stated that nutritional content is a factor that supports egg quality, including high egg white. Another factor that affects egg white height is the length of egg storage. FITRA et al. (2020) stated that egg white height was influenced by the length of egg storage. The eggs produced in this study were immediately observed. Egg white height will decrease due to storage for too long because it affects the function of ovomucin and egg white viscosity which is characterized by high egg white thickness. The high number of egg whites in P3 did not increase, due to the high fiber in cassava leaf paste resulting in low feed consumption. Cassava leaf paste contains 1.87% crude fiber (JUMADIN et al. 2022a)

The high number of egg yolks in this study was not statistically significantly different. Treatment P2 obtained the highest egg yolk height value with a value of 12.35 mm. The high number of egg yolks was due to differences in nutrient content between treatments. JUMADIN et al. (2022a) stated that nutritional content is a factor that supports egg quality, including high egg yolk. Another factor that affects egg yolk height is the length of egg storage. FITRA et al. (2020) stated that the yolk index including egg yolk height was influenced by the length of egg storage. In this study, the eggs produced were immediately observed.

Egg composition

Observations of egg composition in this study included calculating the percentage of egg shells, the percentage of egg whites, and the percentage of egg yolks. The results of these observations are presented in Table 3.

Table 3

Composition of quail eggs given cassava leaf paste for 30 days

Variable	P0	P1	P2	P3
Egg shell [%]	11.91 ±0.91	12.23 ±1.74	10.95 ±0.75	11.64 ±1.19
Egg whites [%]	54.43 ±5.60	61.24 ±13.20	61.59 ±4.76	65.07 ±12.75
Egg yolk [%]	36.30 ±3.03	36.92 ±2.28	38.39 ±4.38	36.74 ±1.31

Numbers accompanied by different letters in the same row indicate significantly different ($p < 0.05$); P0 – cassava leaf paste 0 mg/g; P1 – cassava leaf paste 5.29 mg/g; P2 – cassava leaf paste 10.58 mg/g; P3 – cassava leaf paste 15.87 mg/g

The results of the analysis of various eggshell percentages showed that there was no effect on various levels of cassava leaf paste administration. Treatment P1 obtained the highest percentage of eggshell with a percentage value of 12.23% of eggshell. This is due to differences in nutrient content and mineral elements between treatments. ABOU-ELKHAIR et al. (2020) stated that the nutritional content and mineral elements are factors that support the percentage value of quail egg shells. Another factor that affects the percentage of eggshells is differences in flavonoids in cassava leaf paste. ABDEL-WARETH and LOHAKARE (2021) and CUI et al. (2020) stated that the increase in egg weight including egg shells was due to the presence of flavonoids which can increase the storage of glucose, phosphorus, calcium, zinc, and iron into egg cells.

The percentage of quail egg whites receiving cassava leaf paste was higher than the control treatment. The cause of the high percentage of egg whites is due to differences in nutritional content, mineral elements, and flavonoids in cassava leaf paste. ABDEL-WARETH and LOHAKARE (2021) and CUI et al. (2020) stated that the increase in egg weight including egg white was due to the presence of flavonoids which can increase the storage of glucose, protein, phosphorus, calcium, zinc, and iron into the oocyte. Cassava leaf paste contains high crude protein and low crude fiber.

The different levels of cassava leaf paste in this study also did not have a significant effect on the percentage value of egg yolks. The percentage of quail egg yolks receiving cassava leaf paste was higher than the control treatment. The high percentage value of egg yolks is due to differences in the nutritional content, mineral elements, and flavonoids in cassava leaf paste. Flavonoids can reduce oxidative stress so that the deposition of glucose, triglycerides, cholesterol, phosphorus, calcium, zinc, and iron into egg cells is more efficient (VAN DE WIER et al. 2017, CUI et al. 2020, ABDEL-WARETH and LOHAKARE 2021).

Conclusion

Quail with cassava leaf paste treatment resulted in quail performance such as body weight gain, and higher egg mass than without cassava leaf paste treatment. Giving cassava leaf paste can cause quail to be healthier. The quails that were given a dose of cassava leaf paste of 5.29 mg/g (P1), 10.58 mg/g (P2), and 15.87 mg/g (P3) produced thicker egg shells than the group of quail eggs treated with cassava leaf paste didn't get the cassava leaf paste. Quail with cassava leaf paste treatment resulted in higher egg compositions such as egg white and yolk percentage than without cassava leaf paste treatment.

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INTERSEXUAL DIFFERENCES IN BODY TRAITS IN SELECTED COCKROACH SPECIES*

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Abstract

In cockroaches sexual dimorphism in body size is common and all patterns are exhibited, however female size bias predominates. The aim of present study was to evaluate intersexual differences in body dimensions and weight in 12 cockroach species: *Blaberus craniifer* Burmeister, *Blaberus discoidalis* Serville, *Blaberus boliviensis* Princis, *Eublaberus posticus* (Erichson), *Eublaberus distanti* (Kirby), *Archimandrita tessellata* Rehn, *Blaptica dubia* (Serville), *Panchlora nivea* (L.), *Nauphoeta cinerea* (Olivier), *Phoetalia pallida* (Brunner von Wattenwyl), *Shelfordella lateralis* (Walker) and *Periplaneta americana* (L.). Fifty male and 50 female adults of each species were weighted and their body length, length and width of pronotum and lengths of antennae and tegmina were measured. In all species female-biased sexual size dimorphism was present: females were significantly heavier and their pronota were longer and wider. Also females body length had higher values in 10 out 12 species. In other two species intersexual differences in body length appeared to be insignificant. On the other hand, male antennae were significantly longer in majority of studied species, with exception of *P. nivea*, *P. pallida* and *N. cinerea*. Thus, in majority of studied species, males had longer antennae in spite of their smaller sizes.

Introduction

Cockroaches inhabit various natural and anthropogenic habitats. Most species of cockroaches dwell in the tropical or subtropical areas (HUTCHINS et al. 2003). They make up to 24% of the today's tropical forests

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canopies biomass (BASSET 2001). On the other hand, dozens of species of cockroaches are able to adapt to live in human-changed environment.

In the natural environment cockroaches are important organisms as detritivores (NALEPA et al. 2001, SCHEU and SETALA 2002). They feed on dead vegetation, animals and excrements (ROTH and WILLIS 1957), thereby maintaining a balance in the ecosystem. They may be the part of the food chain as nourishment for insectivorous plants, other insects, amphibians, reptiles and mammals (BOROSSUT 1983).

Many cockroach species are able to colonize different environments. In addition to their natural environments as forests, grassland and caves, they are perfectly adapted to anthropogenic environments. In most cases, man is responsible for spreading cockroaches to new environments (ROTH and WILLIS 1960).

Most people perceive cockroaches as negative figures in the world of insects. It is partly justified because they carry pathogens, parasites, etc. They also cause damage or contaminate food. However, only some species of this group can be threat to humans. People take actions to control species considered as pests such as *Periplaneta americana*, *Blatta orientalis*, *Blatta germanica* and *Nauphoeta cincerea* (HILL 2002). Despites many concerns, cockroaches are increasingly selected as model animals in many studies and projects (HUBER et al. 1990). The studies on cockroaches are focused on morphology, physiology, behaviour, evolution and pathogen transmission (BELL 1981). These insects are characterized by easy adaptation to new condition, fast growth, high fertility, vitality and are omnivorous (SCHAL et al. 1984).

The above mentioned facts increase interest in cockroaches in many areas, they are used as feed for exotic animals and kept as pets.

Characteristics that are relevant to determine sexual dimorphism can be the size, shape and colour of particular body parts such as the head (antennae, composed eyes), thorax (pronotum, hindwings, forewings, tegmina, legs) or abdomen (tergum, sternum, subgenital plate, styli, cerci) (BELL et al. 2007). Measurement of body length used in research sometimes depends on wings presence. Some authors measure body length from top of the head to the end of abdomen, while others measures form the beginning of the pronotum to the end of the wings. Sexual dimorphism in body size (sexual size dimorphism – SSD) is common in cockroaches and all patterns exist, but female size bias predominates (BELL et al. 2007). Depending on which sex is larger, it is defined as female-biased sexual size dimorphism (female-biased SSD) or male-biased sexual size dimorphism (male-biased SSD) (BLANCKENHORN et al. 2009). Females of cockroaches often are heavier, and have larger abdomen, sometimes incubate ootheca and acquire a greater weight in preparation for pregnancy. It is worth to notice that adult body mass largely depends on diet and availability of food during larval development.

Although cockroaches attract interest of scientists and one can find very good sources of knowledge of this insect group (e.g. BELL et al. 2007), there is lack of biometric data of some (many?) species, even popular ones. DJERNÆS et al. (2020) provided (as electronic supplement files) tables with body and pronotum lengths of males and females of many Blaberoidea species, but antennae and tegmina lengths were not given (only information if males and females of a given species are apterous, brachypterous or macropterous).

The aim of this study was to create a biometric data of 12 species. Greater attention was directed to these parts of the cockroaches body, which are particularly significant in recognising their sex. Measurements of specific body parts of insects were made to determinate whether sexual dimorphism of these species was observed. The study was performed on twelve species of cockroaches. Within the family Blaberidae there have been selected: *Blaberus craniifer* Burmeister, *Blaberus discoidalis* Serville, *Blaberus boliviensis* Princis, *Eublaberus posticus* (Erichson), *Eublaberus distanti* (Kirby), *Archimandrita tessellata* Rehn, *Blaptica dubia* (Serville), *Panchlora nivea* (L.), *Nauphoeta cinerea* (Olivier) and *Phoetalia pallida* (Brunner von Wattenwyl). The other two species belong to the Blattidae family: *Shelfordella lateralis* (Walker) and *Periplaneta americana* (L.).

Species were selected based on their availability and usefulness for the experiment. Moreover, species such as *P. americana*, *N. cinerea*, *B. discoidalis* and *S. lateralis* gained attention among scientist and breeders. They are considered as model insects for experiments and research (HUBER et al. 1990).

Materials and Methods

Twelve species of cockroaches: *Blaberus craniifer*, *Blaberus boliviensis*, *Blaberus discoidalis*, *Eublaberus distanti*, *Eublaberus posticus*, *Archimandrita tessellata*, *Blaptica dubia*, *Panchlora nivea*, *Phoetalia pallida*, *Nauphoeta cinerea*, *Shelfordella lateralis* and *Periplaneta americana* were studied. Fifty males and females from each species were selected for measurement. After a last moulting, imago forms were separated from the rest of the group, to exclude the possibility of fertilization, food consumption or possible injuries caused by other individuals, and in order to standardize measurements.

All above mentioned species were kept in incubator with a daily cycle: 12 hours of light at temperature of 28°C and 12 hours of dark at temperature of 24°C. They were kept in plastic containers (dimensions: 35cm x 30cm x 15cm) with upper ventilation, water was added to containers (spraying). Coconut fibers were used as a litter, cartoon egg trays were provided as harborages for some species (*Nauphotea cinerea*, *Blaptica dubia*, *Phoetalia pallida*, *Shelfordella lateralis*, *Periplaneta americana*). All species were fed with the same food: fruits, vegetables (mainly apples and carrots) and dog food. Cockroaches were caught every three days and measured on the same day or the day after exoskeleton hardened. Individuals' sex was determined by subgenital plate size measurement or (in *B. dubia* and *S. lateralis*) wings presence. Only adults without external damages or deformities (e.g. lost leg or antennae) were included in our study.

The following measurement were made:

- length of antennae, measured from the scape to the end of flagellum;
- length and width of pronotum, measured at the most extended part;
- tegmina length, measured from the end of pronotum to the end of tegmina;
- body length, measured from the head beginning to the end of abdomen. This method of measuring body length was used due wing reduction in some of studied animals;
- body weight.

All measurements were performed by the same person (A.P.) using the same grasp involving hold on to the sides of cockroach's body, so that there was no increase or decrease of the abdomen. With this grasp insects were able to freely maintain the abdomen. Electronic calliper (Swiss Precision Instruments) and electronic weighing scales (Diamond model 100) were used. ANOVA was performed to asses potential intersexual differences.

Results

All means and standard deviations of taken measurements are presented in Table 1.

Out of 12 selected cockroach species, *Archmandrita tessellata* was the largest and the heaviest. *Panchlora nivea* have the smallest males and *Phoetalia pallida* have the smallest females.

It was also shown that statistically significantly larger (body length) in majority of species were females. Additionally, females always had longer and wider pronotum, and were heavier. We stated that tegmina were significantly longer in females of seven studied species, but in five studied species males had longer tegmina (females of *B. dubia* and *S. lateralis* have reduced wings). On the other hand, males usually have longer antennae but in three small species females had significantly longer antennae. Most of differences were statistically significant, only body length differences in *Blaptica dubia* and *Periplaneta americana* were not significant (Tables 1, 2).

Table 1

Body dimensions and weight of males and females: means and (SD). Fifty males and 50 females of every species were measured

Species	Traits											
	Body length [mm]		Pronotum width [mm]		Pronotum length [mm]		Antennae length [mm]		Tegmina length [mm]		Body weight [g]	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	F
B. craniifer	45.63 **(2.64)**	49.11* **(2.66)**	16.48 **(0.70)**	18.81* **(0.75)**	11.84 **(0.44)**	13.19* **(0.61)**	32.96 **(2.61)**	30.34* **(3.40)**	45.48 **(1.66)**	48.07* **(1.88)**	2.49 **(0.41)**	3.63* **(0.52)**
B. discoidalis	40.26 **(2.53)**	44.33* **(2.69)**	15.23 **(0.70)**	17.17* **(0.79)**	10.51 **(0.39)**	11.47* **(0.46)**	25.11 **(2.41)**	23.66* **(2.21)**	35.14 **(1.54)**	35.81* **(1.60)**	1.78 **(0.21)**	2.83* **(0.34)**
B. boliviensis	39.42 **(2.67)**	42.30* **(1.96)**	15.17 **(0.58)**	16.57* **(0.63)**	10.43 **(0.41)**	11.27* **(0.36)**	25.55 **(2.17)**	24.40* **(1.37)**	35.28 **(1.21)**	34.30* **(1.67)**	1.80 **(0.17)**	2.68* **(0.26)**
E. posticus	42.22 **(3.16)**	46.39* **(3.18)**	16.25 **(1.09)**	17.56* **(0.89)**	11.55 **(0.67)**	12.34* **(0.52)**	30.07 **(2.63)**	28.16* **(1.71)**	35.37 **(1.75)**	38.89* **(1.76)**	2.70 **(0.53)**	3.86* **(0.76)**
E. distanti	44.83 **(3.46)**	46.83* **(2.27)**	16.50 **(0.81)**	17.00* **(0.72)**	11.68 **(0.61)**	11.92* **(0.58)**	26.17 **(1.89)**	23.78* **(1.82)**	38.02 **(1.40)**	40.70* **(1.38)**	3.83 **(0.54)**	4.68* **(0.57)**
A. tessellata	54.30 **(3.08)**	57.98* **(2.03)**	24.20 **(0.90)**	26.94* **(0.95)**	15.08 **(0.52)**	16.36* **(0.55)**	34.22 **(2.35)**	28.84* **(2.54)**	50.60 **(2.09)**	47.08* **(1.90)**	4.80 **(0.62)**	7.21* **(0.75)**
B. dubia	34.08 **(1.84)**	33.92 **(2.19)**	12.26 **(0.52)**	14.87* **(0.69)**	8.70 **(0.42)**	9.64* **(0.41)**	19.98 **(2.32)**	17.58* **(2.23)**	32.44 **(1.08)**	10.01* **(0.88)**	1.27 **(0.17)**	1.74* **(0.26)**
P. nivea	12.90 **(0.90)**	18.74* **(1.45)**	4.58 **(0.33)**	6.60* **(0.59)**	3.69 **(0.38)**	5.08* **(0.36)**	11.07 **(0.93)**	12.94* **(1.17)**	14.53 **(0.85)**	20.17* **(1.03)**	0.08 **(0.02)**	0.25* **(0.05)**
N. cinerea	23.44 **(1.89)**	25.73* **(1.48)**	7.72 **(0.38)**	8.30* **(0.44)**	5.44 **(0.26)**	5.82* **(0.34)**	19.87 **(1.44)**	20.75* **(1.18)**	16.82 **(0.69)**	19.67* **(0.96)**	0.44 **(0.05)**	0.56* **(0.07)**
P. pallida	15.55 **(0.80)**	18.63* **(0.93)**	4.99 **(0.23)**	6.07* **(0.26)**	3.22 **(0.25)**	3.89* **(0.23)**	11.73 **(0.87)**	12.49* **(0.93)**	13.58 **(0.52)**	15.57* **(0.61)**	0.13 **(0.01)**	0.24* **(0.03)**
S. lateralis	21.48 **(1.32)**	22.98* **(1.27)**	6.18 **(0.32)**	7.53* **(0.39)**	4.85 **(0.39)**	5.72* **(0.34)**	24.96 **(3.91)**	21.62* **(2.51)**	21.97 **(1.34)**	5.62* **(0.45)**	0.28 **(0.04)**	0.45* **(0.08)**
P. americana	30.82 **(1.68)**	29.86 **(3.56)**	9.11 **(0.46)**	9.61* **(0.57)**	7.33 **(0.37)**	7.67* **(0.36)**	47.13 **(4.37)**	42.26* **(4.32)**	30.26 **(1.71)**	25.75* **(1.77)**	0.87 **(0.11)**	0.94* **(0.20)**

* Significantly different between males and females (ANOVA, $P < 0.05$)

Table 2

Comparison of biometrical traits between sexes (F-female biased, M-male biased

Species	Body length	Pronotum width	Pronotum length	Antennae length	Tegmina length	Body weight
B. craniifer	F*	F*	F*	M*	F*	F*
B. discoidalis	F*	F*	F*	M*	F*	F*
B. boliviensis	F*	F*	F*	M*	M*	F*
E. posticus	F*	F*	F*	M*	F*	F*
E. distanti	F*	F*	F*	M*	F*	F*
A. tessellata	F*	F*	F*	M*	M*	F*
B. dubia	M	F*	F*	M*	M*	F*
P. nivea	F*	F*	F*	F*	F*	F*
N. cinerea	F*	F*	F*	F*	F*	F*
P. pallida	F*	F*	F*	F*	F*	F*
S. lateralis	F*	F*	F*	M*	M*	F*
P. americana	M	F*	F*	M*	M*	F*

*difference significant at p level <0.05), letter indicates sex with bigger value of the trait

Discussion

We selected cockroaches which represent two families (Blaberidae and Blattidae) in this study. These species were chosen due to their availability and ease to perform biometric measurements.

Occurrence, reduction or absence of certain body parts or their size may indicate the utilisation of adaptation to environmental conditions (MULLINS and COCHRAN 1987). Pronotum has many functions e.g. digging and substrate penetration (SIMPSON et al. 1986) or attacking intruder (SEELINGER and SEELINGER 1983). Using pronotum in establishing hierarchy was observed on males of *Blaberus craniifer* and *Nauphoeta posticus* (EWING 1967, 1972). Bigger pronotum is advantageous during foraging, defending or establishing position in hierarchy. However, it seems that statistically greater pronotum of females in all species in this study may result simply from the overall greater weight and length. Body length was insignificantly greater in males of *B. dubia* and *P. americana* only. In all investigated species females were more massive, since they invest more energy to prepare for reproduction.

"Like many animals active in low-light conditions, cockroaches often use tactile cues to avoid obstacles and guide their locomotion" (BELL et al. 2007). Longer antennae allow better environment investigation and faster reactivity. This is particularly important in the case of males seeking for adult females to mate (e.g. SCHALLER 1978). In this study we showed that most cockroach antennae are statistically longer in males with exception of three small species (*P. pallida*, *N. cinerea*, *P. nivea*) in which females have longer antennae.

DJERNÆS et al. (2020) stated that "Body size in cockroaches ranges from 2.5 mm to 78 mm". Average size and average weight of individuals vary, especially between genders. Males mature earlier than females (ESPERK et al. 2007). Females reach more massive body by more moulting, but this fact also depend on environmental factors (MULLINS and COCHRAN 1987). Development of cockroaches depends on many different factors which influence the SSD such as temperature and humidity. Moreover, cockroaches in groups grow faster and reach more body weight (WOODHEAD and PAULSON 1983), but excessive high density had opposite effect (GOUDEY-PIERRIERE et al. 1992). Damage of the antennae, legs or other body parts slow down development (TANAKA et al. 1987). Cockroaches take care of their offspring, which gives nymphs opportunity to develop faster due to avoidance of predatory attack and creates the best conditions for development during first days of life (PARK and CHOE 2003).

Data presented in this paper confirmed those reported in previous studies on body weight and length of *Blaberus craniifer* (CLARK and TRIBLEHORN 2014, CHOATE 2003, GERE 1985). Also data collected from measurements of *Periplaneta americana* (CLARK and TRIBLEHORN 2014, ROTH 2003) did not differ from our results. However, DAY (1950) reported a higher body mass for individuals that had no possibilities to get food or produce ootheca. Body length of *Nauphoeta cinerea* presented by BELL (1981) and ROTH (2003), *Panchlora nivea* given by DJERNÆS et al. (2020, after Hebard 1919) and *Eublaberus distanti* reported by OONINCX and DIERENFELD (2012) are comparable with our results. BELL et al. (2007) have written that; "A male *Archimandrita tessellata* measured by Gurney (1959) stretched to 85 mm", whereas in our study maximum body length of *A. tessellata* (female!) was 61.9 mm. On the other hand, DJERNÆS et al. (2020) reported body length values of *A. tessellata* very similar to those presented here (average for females: 57.01 mm; in our study: 57.98 mm). In case of *Eublaberus posticus* DJERNÆS et al. (2020) also reported that values of body and pronotum length are higher in females, but absolute values given there are lower than in our study (e.g. average female body length 34.1 mm; in our study: 46.39 mm). These differences can probably be explained with the fact that authors mentioned above measured dead, dried specimens. DJERNÆS et al. (2020, after Hebard 1917) showed higher absolute values of body and pronotum length of *Phoetalia pallida* than stated in this paper, but they also stated higher values in females like in our study (e.g. average male and female body length: 16.25 mm and 19.25 mm; in our study respectively: 15.55 mm and 18.63 mm). Our results on *Blaptica dubia* are in agreement with results given by POJASEK (2013): male antennae are longer, females are heavier, female pronotum is significantly longer and wider, but males body length is insignificantly longer. On the other hand, DJERNÆS et al. (2020, after HEBARD 1921) reported that not only females pronotum is longer, but also females body length is bigger (average male and female body length: 32.2 mm and 32.96 mm) – contrary to our result (average male and female body length: 34.08 mm and 32.92 mm).

To the best of authors' knowledge, no report about any measurements has been found for species such as *Blaberus boliviensis*, *Blaberus discoidalis* and *Shelfordella lateralis* so far.

Among insects, SSD is highly influenced by the environmental factors (TEDER and TAMMARU 2005) and further development. Female-biased SSD predominates in cockroaches (BELL et al. 2007) and also is common in invertebrates in general (FAIRBAIRN 2007), e.g. Acrididae (BIDAU et al. 2013), which was also confirmed in this study.

We conclude that sexual dimorphism is present among all of the studied species. Although *Blaptica dubia* and *Shelfordella lateralis* sexual dimorphism is easy to determine (because wings of females are reduced), it has been firmly confirmed in the measurements. Traits such as body weight, pronotum length and width indicate clearly female-biased SSD in all studied species, whereas males of majority of studied species had longer antennae.

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EVALUATION OF ECO-EXTRACTION METHODS OF ANTIOXIDANTS AND THEIR ACTIVITIES FROM *RETAMA RAETAM* TWIGS

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Key words: green extraction, *Retama raetam*, microwave, ultrasound, antioxidant.

Abstract

The purpose of this study is to compare the effectiveness of different conventional and non-conventional methods for the extraction of secondary metabolites and antioxidant activity of aqueous and hydro-ethanolic extract of *Retama raetam* twigs including maceration, reflux, Soxhlet, microwave assisted extraction (MAE) and ultrasonic assisted extraction. The aqueous and hydro-ethanolic extracts obtained by MAE showed the highest contents of total phenolics (160.43 ±1.42 and 175.71 ±2.09 mg EAG/g DR, respectively) and flavonoids of 12.28 ±0.92 and 39.97 ±1.11 mg EC/g DR, respectively. It also exhibited significant DPPH$^{\bullet}$ scavenging capacity with IC_{50} values of 0.45 ±0.075 and 0.34 ±0.039 mg/mL and significant iron reducing capacity with EC_{50} of 0.358 ±0.02 and 0.28 ±0.01 mg/mL for the aqueous and hydro-ethanolic extracts, respectively. The MAE proved to be the most efficient extraction technique for the extraction of antioxidants from *R. raetam* twigs.

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Introduction

Secondary metabolites, such as phenolic components, are commonly found in plants and have a wide range of structures. Furthermore, these compounds are not uniformly distributed in plants and have varying degrees of stability. This has resulted in challenging extraction processes, meaning that the use of a single step or an ineffective extraction approach can affect the recovery of phenolic compounds from plant samples. To recover the desired phenolic components, it is critical to select a suitable extraction procedure. These approaches encompass both conventional and non-conventional ways of extraction (ALARA and ABDURAHMAN et al. 2021). However, microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) are among the non-conventional green chemistry (GC) extraction methods (ZIN and ANUCHA et al. 2020). GC is the need of today and the light of the future which gives a precious idea for scientifically based environmental protection (ASIF and IMRAN 2021). It is a fascinating research area due to its respect for the environment (MENGES 2021) GC involves a reduction or elimination of the use of hazardous substances in a chemical process or the generation of hazardous or toxic intermediates or products. This includes feedstock, reagents, solvents, products, and byproducts. It also includes the use of sustainable raw materials and energy sources for this manufacturing process (DOBLE and KRUTHIVENTI 2007). MAE is considered a new method of extracting fluid soluble products from many materials using microwave energy (REHMAN and KHAN et al. 2020). it is an automated green extraction technique that offers many advantages such as faster heating to extract bioactive materials from matrices, smaller equipment size, reduced thermal gradients and the possibility to extract several samples simultaneously, thus considerably improving the sample throughput and increasing extract yield (LLOMPART and CELEIRO et al. 2019). The extraction time of bioactive compounds in the case of MAE is lower than conventional extraction methods. In addition, it is a selective process for the extraction of organic and organometallic substances (REHMAN and KHAN et al. 2020). While, ultrasound-assisted extraction is another effective technique that has become more popular since 2007 (REDDY and MONIRUZZAMAN et al. 2020). UAE has been considered a promising and innovative technique with many applications in the chemistry, pharmaceutical, cosmetic, and alimentary fields of the 21st century (CHAHARDOLI and JALILIAN et al. 2020). It is also used in the search for bioactive compounds as it is based on the effects of acoustic cavitation. The propagation of ultrasonic waves allows greater penetration of the solvent into the sample matrix, which increases the contact between the sample and the solvent and improves mass transfer rates (DUARTE and JUSTINO et al. 2014). To the best of our knowledge, no study has been conducted on the extraction of secondary metabolites from the *R. raetam* plant using GC techniques. Therefore, the present study was conducted to investigate the efficiencies of MAE and UAE methods as innovative and eco-friendly technology using two green solvents compared to conventional extraction techniques such as maceration, reflux and Soxhlet for the extraction of major secondary metabolites and evaluation of their antioxidant activities.

Materials and Methods

Chemicals and reagents

Analytical grade ethanol, 2-(3,4 Dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin; C), 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 3,4,5-trihydroxybenzoic acid (Gallic acid; GA), 3-methoxy-4-hydroxybenzaldehyde (Vanillin), aluminum chloride ($AlCl_3$), ammonium molybdate ($(NH_4)_6Mo_7O^2{}_4$), Folin-Ciocalteu phenol reagent, hydrochloric acid (HCl), iron chloride ($FeCl_3$), potassium ferricyanide solution $K_3Fe(CN)_6$, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), and sulfuric acid (H_2SO_4). All chemicals used were obtained from either Sigma-Aldrich (Madrid, Spain) or Merck (Darmstadt, Germany).

Plant sampling

Samples of the *R.raetam* twigs were collected in February 2019 during the flowering period in the region of Bousemghoun, El Bayadh-Algeria (Latitude: 32.8643, longitude: 0.02012 32°51′51″ North, 0°1′12″ East). The plant used was identified by the botanist Amar Eddoud (Department of Biology at the Kasdi Merbah University of Ouargla-Algeria). The twigs of the plant were dried in amber and then ground into a fine powder for later use.

Preparation of plant extracts

The extraction of secondary metabolites was performed using five different extraction techniques including maceration, reflux, Soxhlet, MAE, and UAE. The extraction yields were calculated using the following formula (1):

$$\text{Yield of extract [\%]} = \frac{\text{weight of extracts from plant sample [g]}}{\text{weight of dried plant sample [g]}} \cdot 100\% \qquad (1)$$

Conventional extraction techniques

The maceration method was performed by continuously stirring the mixture of plant powder (10 g) with 100 mL of solvent (water or ethanol 70%) at room temperature for 24 hours in the dark. The same solvent/sample ratio was boiled at 100°C in a 100 mL water bath reflux system for 15 min for heat reflux and Soxhlet extraction. The obtained mixtures were filtered with N°1 Whatman, and centrifuged at 3000 rpm for 5 min. The extracts were then delipidated with a petroleum ether solvent and evaporated to dryness in a rotary vacuum evaporator. The resulting residues were stored at 4°C until use.

MAE and UAE process

The MAE was carried out according to the protocol described by (Olalere and Gan 2021). 10 g of plant powder was added to 100 mL of different solvents (water, ethanol 70%). The mixture was irradiated for 10 min in a discontinuous process using a model microwave apparatus that operates at a frequency of 2450 kHz. The microwave equipment has been modified to condense the vapors generated during extraction. Concerning the UAE, it was carried out according to the method described (Nguyen Tram Anh and Van Hung et al. 2021), by using an ultrasonic bath at a frequency of 35 kHz, power of 20 W.

The obtained mixtures were filtered with N°1 Whatman and centrifuged at 3000 rpm for 5 min. The extracts were then delipidated with a petroleum ether solvent and evaporated to dryness in a rotary vacuum evaporator. The resulting residues were stored at 4°C until use.

Assessment of phytochemicals

The assessment of total polyphenol content (TPC) in different extracts was determined using the Folin-Ciocalteu spectrophotometric assay according to (Serairi-Beji and Wannes et al. 2018). TPC in the sample was calculated as milligrams of gallic acid equivalents per gram of dry residue (mg GAE/g DR). The evaluation of total flavonoid content (TFC) was measured by using the $NaNO_2$-$Al(NO_3)_3$-NaOH system (Dewanto and Wu et al. 2002) with minor modifications. It was expressed as milligrams of (+)-catechin equivalent per gram of dry residue (mg CE/g DR). The estimation of total tannin content (TTC) was assayed using the method of (Serairi-Beji and Wannes et al. 2018) with minor modifications. It was expressed as milligrams (+)-of catechin equivalents per gram of dry residue (mg CE/g DR).

DPPH• scavenging activity

The method described by (SÁNCHEZ-MORENO and LARRAURI et al. 1998) was used to measure the DPPH• scavenging activity. 50 µL of each extract at different concentrations (from 0.078 to 5 mg/mL) were added to 1.95 mL of DPPH• methanolic solution (0.025 g/L). Simultaneously, a negative control was prepared by combining 50 µL of methanol with 1.95 mL of DPPH• methanolic solution. The mixture was briskly shaken before being allowed to stand at room temperature for 30 minutes in the dark, at 515 nm. The absorbance of the resulting solution was measured. The scavenging activity was represented as IC_{50} mg/mL, which is the dose necessary to inhibit DPPH• by 50%. The percentage of DPPH• scavenging was calculated according to the following equation (2):

$$\text{DPPH} \bullet \ \text{scavenging [\%]} = \frac{(\text{ABS0} - \text{ABS1})}{\text{ABS0}} \cdot 100 \qquad (1)$$

ABS0 = absorption of control negative (DPPH solution without extract).
ABS1 = absorption of the sample.

Ferric reducing antioxidant power assay (FRAP)

The FRAP is determined according to the method described by (WU and SUN et al. 2014). 1 mL of the extract was combined with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a potassium ferricyanide solution $K_3Fe(CN)_6$. For 20 minutes, the mixture is incubated in a water bath at 50°C. After that, 2.5 mL of 10% trichloroacetic acid is added to halt the reaction, and the tubes are centrifuged for 10 minutes at 3000 rpm. A portion of the supernatant (2.5 mL) is mixed with 2.5 mL of distilled water and 0.5 mL of an aqueous 0.1 percent $FeCl_3$ solution. The absorbance of the reaction medium is measured at 700 nm against a similarly prepared blank, with the extract replaced by solvent, allowing the device to be calibrated (UV-VIS spectrophotometer).

Statistical Analysis

In the present study, all the trials were performed three times and their results were expressed as mean ± Standard Error of the mean and analyzed using the Sigma-Plot version 11.0 program. Statistical analyses were performed by analysis of variance ANOVA (Anova One way), followed by Tukey's test. The difference was considered statistically significant when $p < 0.05$ compared to the negative control. IC_{50} and EC_{50} values are calculated by a basic and simple method based on nonlinear modeling between X (concentrations) and Y (response) using Origin Pro program version 2016.

Results and Discussion

Phytochemical assessment

Green chemistry is the need of the hour and the light of the future, revealing important information for science-based environmental conservation (ASIF and IMRAN 2021). To the best of our knowledge, no previous research has been conducted using GC methods to extract secondary metabolites from *R. raetam* twigs. For this reason, the purpose of this study was to compare the effectiveness of MAE and UAE methods as an innovative and eco-friendly technology to conventional extraction techniques including maceration, reflux, and Soxhlet.

The different extracts obtained were evaluated for their levels of yield, TPC, TFC and TTC. The results of this evaluation are summarized in Table 1.

Table 1

Yield%, TPC, TFT, and TTC of crude aqueous and ethanol extracts of *R. raetam* twigs

Method	Extraction yield [%]		TPC [mg GAE/g DR]		TFT [mg CE/g DR]		TTC [mg CE/g DR]	
	H_2O	EtOH 70%	H_2O	EtOH 70%	H_2O	EtOH 70%	H_2O	EtOH 70%
Maceration	18.89^a	14.68^c	155.13 ±1.7^c	106.51 ±1.89^d	8.69 ±0.88^d	31.51 ±1.28^c	9.68 ±1.87^b	15.17 ±2.22^a
Reflux	13.10^c	9.80^e	120.77 ±2.5^d	136.05 ±1.16^b	11.25 ±1.17^d	25.61 ±1.17^c	2.80 ±1.87^e	5.17 ±1.55^d
Soxhlet	11.64^a	12.40^d	142.6 ±2.76^b	136.17 ±1.89^b	9.20 ±1.55^e	22.79 ±2.46^d	6,15 ±1.53^c	10.27 ±2.04^b
M A E	14.71^b	20.64^a	175.71 ±2.09^a	160.43 ±1.42^a	12.28 ±0.92^b	39.97 ±1.12^a	13.41 ±3.05^a	14.78 ±2.72^a
U A E	12.87^c	16.45^b	146.17 ±2.2^b	111.91 ±1.49^c	15.61 ±1.93^a	32.28 ±1.56^b	4.98 ±2.04^d	6.94 ±1.22^c

Explanations: Results are shown as mean ± Standard Error of the Mean (SEM). Comparison between groups was made using Tukey's test. Columns not sharing a common letter (*a*–*e*) differed significantly at $p < 0.05$

According to the reported findings, the highest yield obtained using water as solvent was 18.89% for maceration. following in decreasing order, by MAE (14.71%), reflux (13.17%), UAE (12.87%) and lastly the Soxhlet (11.64%). While, as for the hydro-ethanol mixture, the highest yield was obtained by the MAE method (20.64%) followed by UAE (16.45%), maceration (14.68%), Soxhlet (12.4%) and lastly reflux (9.8%). According to these results, the maceration technique yielded the best yield when using water as a solvent. The MAE process yielded the best yield when utilizing hydro-ethanolic solution as a solvent. In some previous studies, extraction yields for *R.raetam* twigs were reported to be between 15% and 20%, using maceration of the aerial part of the plant in water and methanol (CONFORTI and STATTI et al. 2004, ALGHAZEER and EL-SALTANI et al. 2012, DJEDDI and KARIOTI et al. 2013).

The significantly higher TPC was recorded for the aqueous extracts ranging from 106.51 ±1.89 mg GAE/g DR to 175.71 ±2.09 mg GAE/g DR for maceration and MAE, respectively. The use of hydro-ethanolic solvent resulted in the highest TPC for MAE with a value of 175.71 ±2.09. These results are in agreement with those of (MARIEM and HANEN et al. 2014) as well as (ALGHAZEER and EL-SALTANI et al. 2012). Where their reported values were 137 and 89.35 ±2.1 mg GAE/g DR, respectively. However, our results are higher than those reported in the study of (SAADA and FALLEH et al. 2018) (27.75 ±0.02 mg GAE/g DR) and (DJEDDI and KARIOTI et al. 2013) (25.19 mg GAE/g DR). In their studies, they used conventional extraction methods such as maceration and Soxhlet for the aerial part of *R. raetam* using water and hydro-methanolic solvent, respectively.

The assessment of TFC revealed that the two non-conventional extraction methods (MAE and EAU) recorded the highest levels with values of 12.28 ±0.92 and 15.61 ±1.93 mg EC/g DR for the aqueous extracts, respectively, and 39.97 ±1.12 and 32.28 ±1.56 mg EC/g DR for the hydro-ethanolic extract, respectively. In comparison, (MARIEM and HANEN et al. 2014) found a TFC of 5.1 mg EC/g DR for the aqueous extract prepared by maceration of the aerial part of the same species. Whereas the TTC estimation indicated that MAE and maceration yielded the best extraction contents with values ranging from 13.41 ±3.05, 9.86 ±1.87 mg EC/g DR for aqueous extracts and 14.78 ±2.72, 15.17 ±2.22 mg EC/g DR for hydro-ethanolic extracts, respectively, for MAE and maceration. The MAE and maceration methods gave the best extraction contents for the two solvents. The results of TTC were not significantly different from those of the study carried out by (MARIEM and HANEN et al. 2014), who reported a content of 10.43 (mg EC/g DR) for the aqueous extract prepared by maceration.

The statistical study showed a highly significant difference ($P < 0.001$) for the different methods employed, solvents and method-solvent interaction. It can be deduced that the nature of the solvent and the extraction method significantly influence the contents of phytochemicals and their antioxidant abilities. This result is in agreement with those reported by several authors (DAHMOUNE and BOULEKBACHE et al. 2013, MANSOURI and LOVILLO et al. 2021). These results are consistent with the findings of (ZAOUI and OUGHLISSI-DEHAK et al. 2021), where the authors indicated that the MAE process of *Calycotome spinosa* allowed obtaining a greater yield when hydro-ethanol was used instead of conventional methods.

This observation could be explained by the important dipole moment of both solvents (BOEING and BARIZÃO et al. 2014) as well as their important dielectric constant (DAHMOUNE and BOULEKBACHE et al. 2013). In this context (CAVALLORO and MARTINO et al. 2021) reported that the use of the mixture (water-organic solvent) can facilitate the extraction of bioactive substances that are soluble in water and/or in the organic solvent. The effectiveness of MAE is certainly due to its mode of action, which is completely different from conventional methods. Microwave irradiation causes a more efficient perturbation of the cell structures, leading to the rupture of the cell wall and membranes. In addition, in the case of a hydroalcoholic mixture combining two polar solvents, the mixture is heated very quickly, which increases its penetration into the matrix, thus facilitating the liberation of the cell contents optimally (AL JITAn and ALKHOORI et al. 2018). The MAE technique is very easy to implement, very fast and requires less solvent than conventional methods. Consequently, it allows to avoid the degradation of thermolabile compounds (DELAZAR and NAHAR et al. 2012, GOURGUILLON and DESTANDAU et al. 2016). In the same context, the study conducted by (LIAZID and PALMA et al. 2007) showed that there is a relationship between the chemical structure and the stability of phenolic molecules during the MAE process. In the case of the UAE technique, it is important to note that the results obtained are not very different from those obtained by conventional methods. The low power and lack of reproducibility of ultrasound applied directly to the sample could be attenuated by the water in the ultrasonic bath and the glassware used for the experiment, as highlighted by (CHEMAT and ROMBAUT et al. 2017). Both the reflux and Soxhlet methods yielded rather lower levels, especially for flavonoids and tannins. The thermal degradation of these compounds during prolonged heating could be the main cause, as confirmed by (SUTAR and GARAI et al. 2010, KARAMI and EMAM-DJOMEH et al. 2015), and (PERVA-UZUNALIĆ and ŠKERGET et al. 2006). Considering the last study, the degradation of catechins was observed at high extraction temperatures (95°C). For the maceration method, it showed moderate results, but its main disadvantage is that it requires several hours of extraction and large amounts of solvent. Furthermore, the study of (ROSELLÓ-SOTO and PARNIAKOV et al. 2016) on the application of non-conventional extraction methods for the sustainable and environmentally friendly production of valuable compounds from mushrooms showed that conventional extraction methods usually involve water or organic solvents and can lead to significant degradation of the constituents, and shows the great potential of these environmentally friendly methods for the eco-friendly production of specific compounds to be used as nutraceuticals or as functional food ingredients.

Antioxidant Activities

DPPH• scavenging activity and FRAP were used to evaluate the antioxidant activity of the different *R. raetam* extracts. The results shown in Figure 1 and Table 2 indicated that the extracts from both solvents with MAE exhibited the highest significant DPPH• scavenging capacity (IC_{50} values of 0.455 ±0.075 mg/mL and 0.34 ±0.39 mg/mL, respectively).

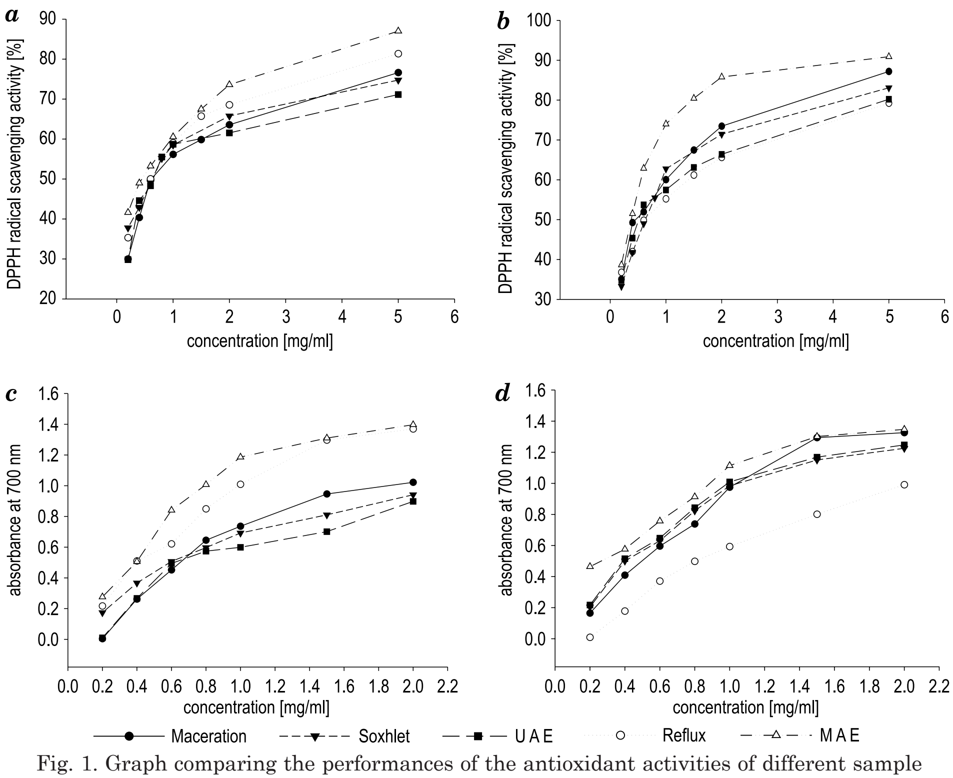

Fig. 1. Graph comparing the performances of the antioxidant activities of different sample extracts of *R. raetam* twigs: *a–b* – DPPH• scavenging activity; *c–d* – FRAP of aqueous and hydro-ethanolic extracts, respectively

In addition, FRAP results showed that the extract prepared by MAE using water and hydro-ethanolic solution had the highest efficiency for reduction (EC_{50} of 0.35 ±0.022 and 0.28± 0.016, respectively). These results are in agreement with the study of (SAADA and FALLEH et al. 2018) (IC_{50} = 0.160 ±0.01 mg/mL) and (HAYET and MAHA et al. 2008)

(IC_{50} = 0.450 mg/mL). Moreover, another study conducted on other species of the genus *Retama* by (BELMOKHTAR and HARCHE 2014) showed that it was significantly able to quench the DPPH• (IC_{50} = 0.15 mg/mL). However, our results were lower than those previously reported by (MARIEM and HANEN et al. 2014) (IC_{50} = 0.043 mg/mL) for the aqueous extract of the aerial part.

Table 2

DPPH• scavenging activity and FRAP of crude aqueous and ethanol extracts of *R. raetam* twigs

Method	IC_{50} for DPPH• test		EC_{50} for FRAP• test	
	H_2O	EtOH 70%	H_2O	EtOH 70%
Maceration	0.72 ±0.04^c	0.52 ±0.04^b	0.61 ±0.03^d	0.49 ±0.04^d
Reflux	0.59 ±0.05^b	0.68 ±0.07^c	0.43 ±0.05^b	0.43 ±0.05^c
Soxhlet	0.70 ±0.06^c	0.55 ±0.060^b	0.57 ±0.03^c	0.35 ±0.03^b
M A E	0.45 ±0.07^a	0.34 ±0.03^a	0.35 ±0.02^a	0.28 ±0.01^a
U A E	0.65 ±0.06^b	0.57 ±0.04^b	0.65 ±0.03^e	0.42 ±0.01^c

Explanations: Results are shown as mean ± Standard Error of the Mean (SEM). Comparison between groups was made using Tukey's test. Columns not sharing a common letter (*a*–*e*) differed significantly at $p < 0.05$

Correlation study

The results presented in Figure 2 show a significant negative correlation between the TPC and the IC_{50} values (R^2 = -0.91) and also with the EC_{50} (R^2 = -0.84). On the other hand, the IC_{50} and EC_{50} values are substantially correlated with an R^2 of about 0.85. The correlation coefficients (R^2 = -0.62, -0.35, -0.72 and -0.57) show that the flavonoid and tannin content of the extracts have a poor relationship with the antioxidant activity. The high values of R^2 obtained in the correlation study shows that polyphenol content and antioxidant activity are strongly correlated. These results are in agreement with many authors (HOSU and CRISTEA et al. 2014, ANJUM and TRIPATHI 2020, KAINAMA and FATMAWATI et al. 2020). It can be concluded that the TPC present in the extracts is mainly responsible for their antioxidant activity. Several authors have suggested that the polar molecules present in plant extracts contribute considerably to increasing their antioxidant activity (CASAGRANDE and ZANELA et al. 2018, NIROULA and AMGAIN et al. 2021). Its activity is due to their ability to release hydrogen (CHIORCEA-PAQUIM and ENACHE et al. 2020).

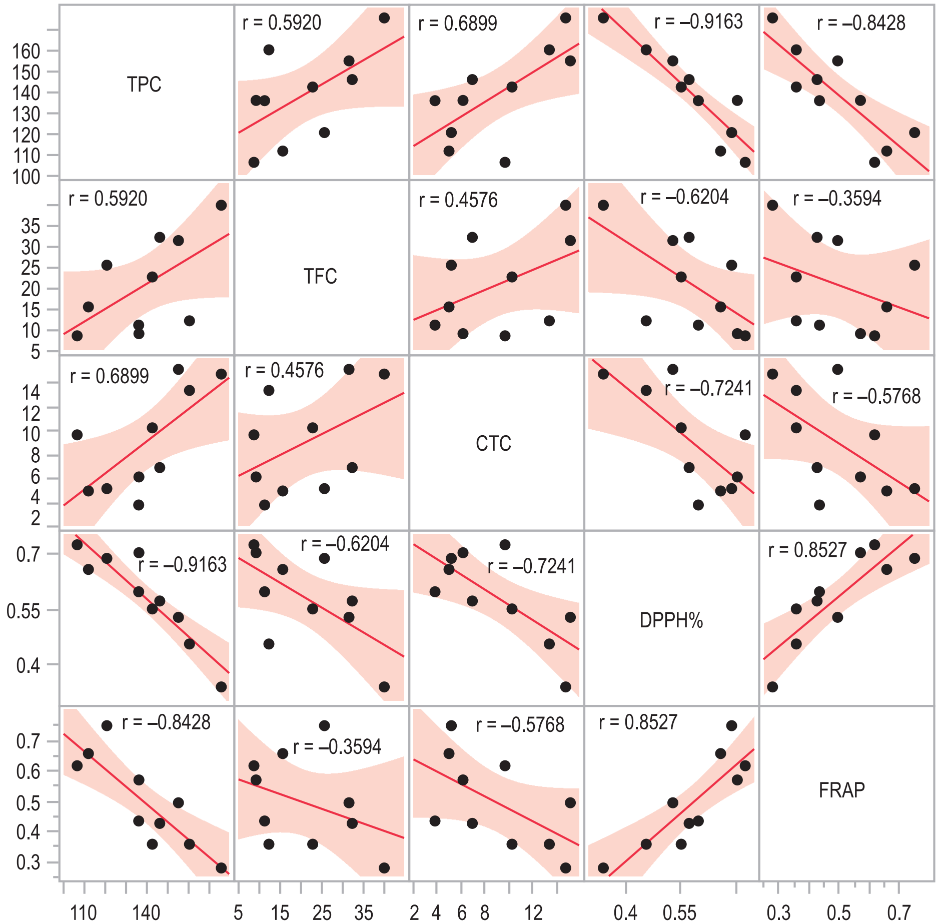

Fig. 2. Correlation matrix between TPC, TFC, TTC, DPPH• scavenging%, and FRAP of different sample extracts of *R. raetam* twigs

Conclusion

The findings of the present investigation indicate that MAE proved to be the most efficient technique yielding the highest levels of phytochemicals obtained from *R. raetam* twigs and exhibiting highly significant antioxidant activity, suggesting that it could be a good source of phytopharmaceutical molecules such as natural antioxidant drugs. Conventional techniques showed much less efficiency as compared to both modern techniques (MAE and UAE).

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EVALUATING SURFACE WATER QUALITY VARIATION IN PHU MY SPECIES-HABITAT CONSERVATION IN KIEN GIANG PROVINCE, VIETNAM

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Key words: acid sulfate soil, conservation area, Phu My, Vietnam, water quality.

Abstract

The study aimed to assess the variation of surface water quality in the Phu My Species-Habitat conservation area (conservation area) in 2019–2022. Fourteen water quality indicators including pH, electrical conductivity (EC), turbidity (Turb), salinity (Sal), total suspended solids (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD), oxygen demand chemical (COD), ammonium (NH_4^+-N), nitrate (NO_3^--N), orthophosphate (PO_4^{3-}-P), total phosphorus (TP), iron (Fe^{2+}) and aluminum (Al^{3+}) were analyzed. Correlation Pearson and cluster analysis (CA) were performed to find the correlation between surface water environmental parameters and locations with similar physical and chemical characteristics of water. The results showed that the surface water in the Phu My conservation area was acidic, with low pH and high concentrations of Fe^{2+} and Al^{3+}. The amounts of organic substances (except COD) and nutrients (NH_4^+-N, NO_3^--N, and PO_4^{3-}-P) in the study area were relatively low. Surface water quality is being well managed to develop key species in the Phu My Species-Habitat conservation area, Lepironia and Eleocharis species. The correlation analysis result indicates that organic matter and nutrients have a positive linear correlation, having the same origin formed from the degradation of organic matter from dying flora and fauna and agricultural activities in the study area. The CA analysis results show that the monitoring system was relatively suitable and could be applied in the future.

Introduction

In Vietnam, wetlands occupy about 12 million hectares, which are irreplaceable in balancing the ecosystem, nutrition, and habitat for many plant and animal species. Wetlands might flood frequently or change sig-

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nificantly in water levels within the area (seasonal wetlands). These areas are strictly managed and protected based on different wetland policies, such as Decree No. 66/2019/ND-CP on the preservation and sustainable use of wetlands (Vietnam Government 2019). However, these lands face various challenges, such as environmental pollution, climate change, or shrinking areas due to encroachment. According to a previous report by Tran et al. (2019), Giang Thanh district, where Phu My is located, will be flooded by up to 99% when the sea level rises by 100 cm.

Phu My Species-Habitat Conservation Area is located at 10°26'41.3" North latitude and 104°36'17.3" East longitude, established in March 2016 to conserve biodiversity in the Long Xuyen Quadrangle, in which priority is given to conservation of the remaining Lepironia grassland in the Vietnamese Mekong Delta. The total land area of the conservation area is 957.87 ha, with three functional areas, including the administrative service area, the ecological restoration area, and the strict protection area. According to Ni (2018), the composition of flora and fauna in the reserve is quite diverse, with more than 456 species recorded, including 47 species of higher plants, 126 species of birds, 30 species of fish, 13 species of reptile and amphibians, 72 species of algae, 67 species of zooplankton, 8 species of benthic animals, 39 species of spiders and 54 species of aquatic insects. However, the number of species in the conservation area tended to decrease over time (Giao 2021). More specifically, the main food source (Eleocharis species) of crane (*Grus antigone sharpii*) – an endangered species listed in the Red Book of Vietnam and the world, is shrinking. One of the most important reasons for declining biodiversity is water environmental quality in the Phu My Species-Habitat conservation area. This has also been evaluated in many previous studies at various water bodies on the impact of water quality on ecosystems and aquatic organisms (Sumandiarsa et al. 2023, Onah 2023). Therefore, water monitoring needs to be conducted regularly and continuously to assess environmental quality promptly, as well as warn of unusual signs affecting the ecosystem's development in the conservation area's water bodies. Nevertheless, there has been no research to evaluate the change in water quality over the years in the conservation area since its establishment. Hence, the present research was carried out to evaluate the water quality changes in the Phu My Species-Habitat conservation area from 2019 to 2022 to provide scientific information for the management and sustainable development of the reserve.

Materials and Methods

Water sampling and analysis

A total of eight surface water samples were collected at locations belonging to the different habitats from 2019 to 2022. Since the conservation area was established management policy based on three functional zones in 2018, the environmental monitoring has been carried out annually until the present. The habitats that have been selected for monitoring include the infield canal, *Lepironia articulata*, Eleocharis, *Melaleuca cajuputi* – Eleocharis, *Melaleuca cajuputi* – *Lepironia articulata*, *Lepironia articulata* – Eleocharis, Peripheral canal (Figure 1). In order to evaluate the characteristics of water quality, water samples were analyzed with fourteen parameters, including pH, electrical conductivity (EC), turbidity (Turb), salinity (Sal), total suspended solids (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium (NH_4^+-N), nitrate (NO_3^--N), orthophosphate (PO_4^{3-}-P), total phosphorus (TP), iron (Fe^{2+}) and aluminum (Al^{3+}). The pH, EC, salinity, and DO parameters were measured directly in the field. Turbidity, TSS, BOD, COD, NH_4^+-N, NO_3^--N, PO_4^{3-}-P, TP, Fe^{2+}, and Al^{3+} were collected, stored, and transported properly and analyzed using standard methods (Apha 1998).

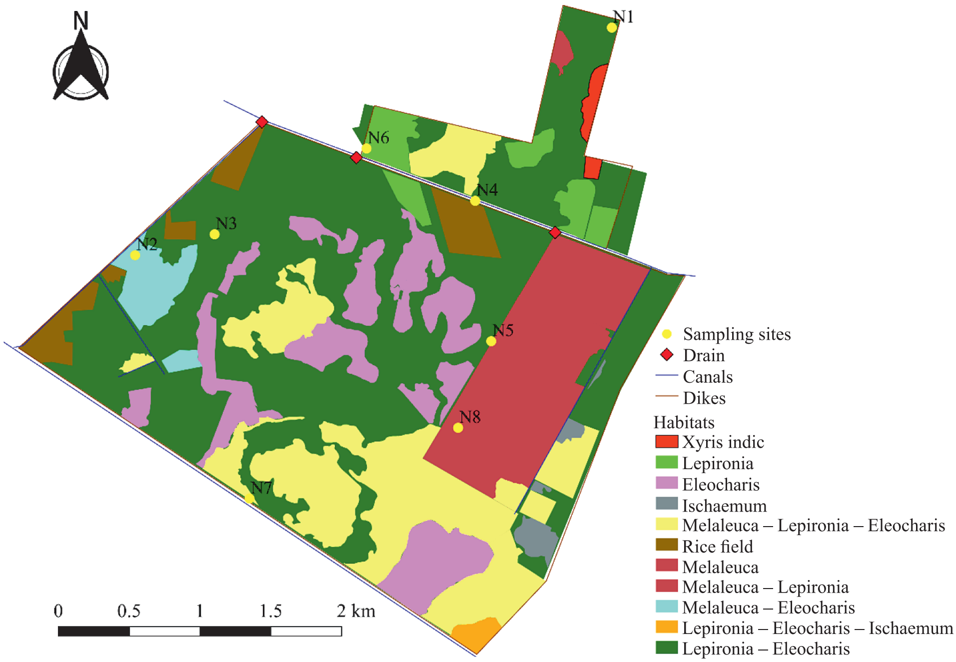


Fig. 1. Map of the sampling locations in the conservation area

Data analysis

The study applied the One-way ANOVA analysis method by using IBM SPSS Statistics for Windows software (version 20.0 IBM Corp., Armonk, NY, USA) to evaluate the statistically significant differences in surface water quality in each water body over the monitoring years 2019– 2022. A Post hoc (Duncan) test was used to show the difference in surface water quality at a 95% significance level ($p < 0.05$). Surface water quality in the study area was compared with national technical regulations on surface water quality (QCVN 08-MT:2015/BTNMT) (Ministry of Natural Resources and Environment in Vietnam 2015). Multivariate statistics such as Pearson correlation analysis and cluster analysis (CA) were performed to find the correlation between environmental indicators affecting surface water quality from 2019 to 2022 and determine suitable monitoring locations.

Results and Discussion

Variation of surface water quality in the reserve during 2019–2022

pH. The analysis found that the pH value in the water bodies of the conservation area fluctuated relatively stable between 2019 and 2022 (Figure 2). The average pH changes in water bodies fluctuated in the range of 3.33 ±0.55–4.68 ±2.58, and the lowest and highest pH were detected in the in-field canals (2020) and rice fields (in 2021), respectively. pH values in the habitats and in-field canals fluctuated between 2.69–4.05 and 2.37–7, while these values in the rice fields and peripheral canals were higher, with about 3.18–8.54 and 3.41–5.52, respectively. pH values gradually increased during the first three years and decreased slightly in 2022 (Figure 2). It can be seen that the pH is acidic; this could be explained by the fact that the soil in this area is acidic soil, which could be suitable for the development of seasonally inundated grassland. Moreover, the previous study of Ni (2018) only has a few species of black fish that are small and capable of living in conditions of heavy alum, low water level, and low nutrition, such as *Anabas testudineus* and other exotic species. pH values were lower than the allowable limit for surface water quality for conserving aquatic plants and animals in column A1 of QCVN 08-MT:2015/ BTNMT (6–8.5). However, this condition was suitable for the growth of Lepironia and Eleocharis, which are key species in the conversation area. Compared with the study of Du et al. (2019), the pH value in water in

U Minh Ha – Ca Mau National Park (5.78 ±0.03–5.83 ±0.78) was also lower than the allowable limit for surface water quality used to conserve aquatic plants and animals. However, the pH in this area is higher than that in the current study area. Whereas the average pH value in the water at Mua Xuan Agriculture Center (Hau Giang province) was higher than in this present study. It creates favorable conditions for aquatic organisms to grow, fluctuating in the range of 6.2 ±0.05–6.7 ±0.01 (DAN et al. 2017). pH profoundly affects the solubility of metals (at low pH), alkalinity, and hardness of water, and aquatic organisms are also affected by pH because of most metabolic activities (WAKAWA et al. 2010).

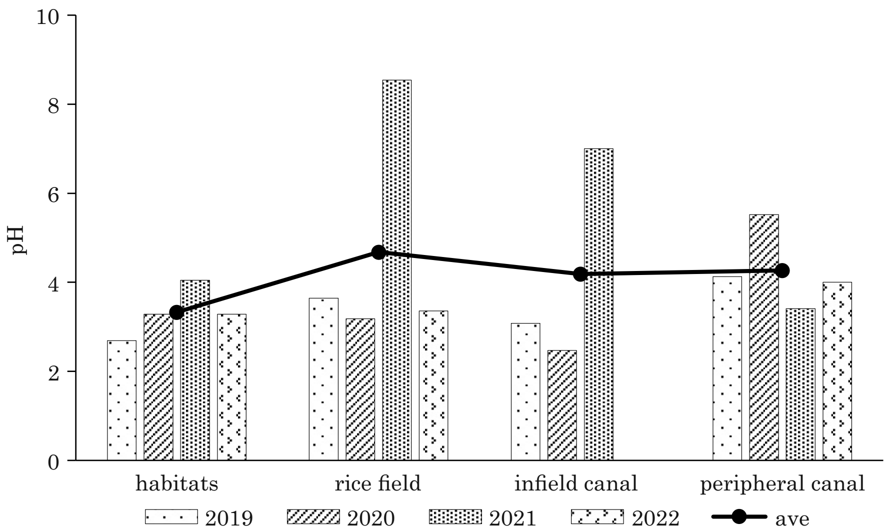


Fig. 2. Variation of pH in the period of 2019–2022 in the conservation area

Electrical conductivity and salinity. Electrical conductivity (EC) is one of the most important water quality parameters for predicting the salinity and mineralization of water (AHMADIANFAR et al. 2020). The results show that EC values and salinity in water in the study area tended to fluctuate similarly from 2019 to 2022, which tended to decrease gradually from 2019 to 2021 and, after that, increased in 2022 (Figure 3). The average value of EC and salinity in water in the water bodies fluctuated between 0.24 ±0.11–0.65 ±0.16 mS/cm and 0.10 ±0.07–0.34 ±0.07‰. The lowest and highest EC values were detected at peripheral canals (in 2020) and the habitats in the reserve (in 2019). EC values in water in habitats, rice fields, in-field canals, and peripheral canals in the period 2019–2022 fluctuated from 0.48–0.85 mS/cm,0.16–0.46 mS/cm, 0.21–0.91 mS/cm and

0.11–0.37 mS/cm, respectively. The salinity in each water body in the period of 2019–2022 ranged from 0.27–0.40‰ (habitats), 0.07–0.20‰ (rice fields), 0.15–0.46‰ (infield canals) and 0.04–0.19‰ (peripheral canals). As a result, it was found that the EC value and the lowest salinity in the peripheral canal could be due to the concentration of ions in the water being diluted in the surrounding canals, leading to a low EC value. The habitats in the reserve have high EC values and salinity because they contain a significant amount of dissolved ions. The difference in EC values and salinity in the study area is mainly due to the presence of dissolved ions present in the acid sulfate soil (GIAO et al. 2020).

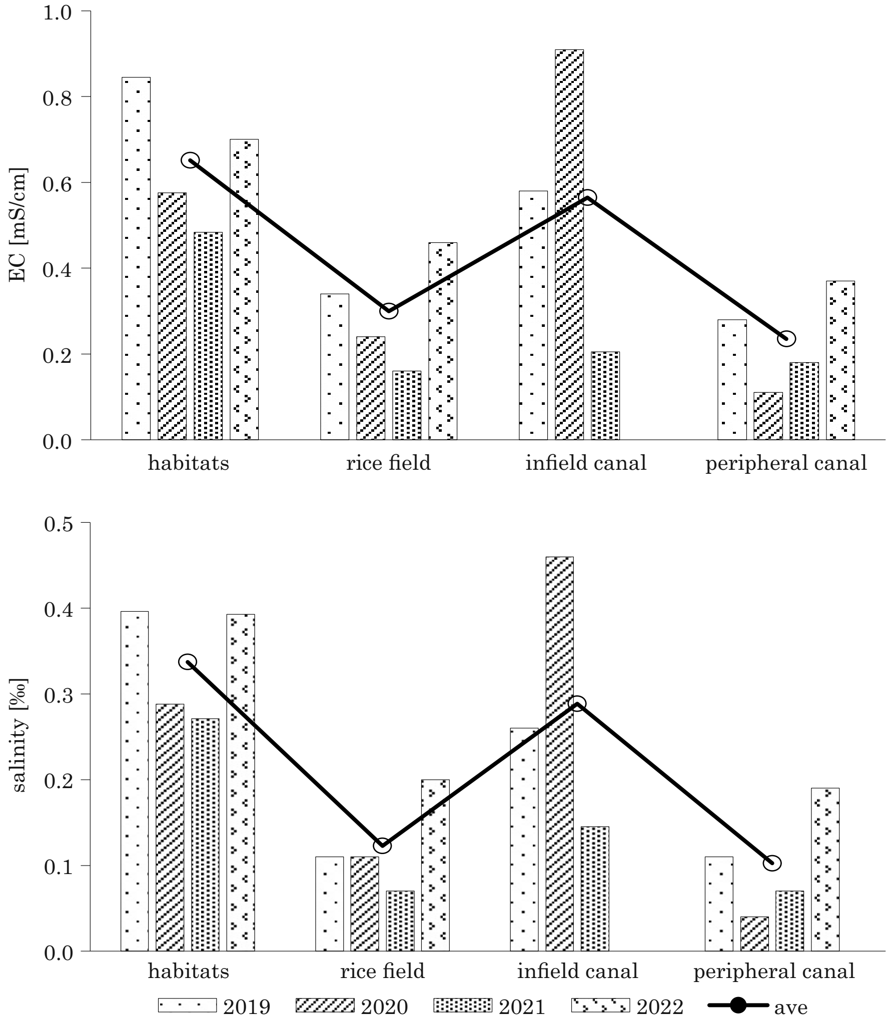


Fig. 3. Variation of EC and Salinity in the period of 2019–2022 in the conservation area

Turbidity and total suspended solids. Turbidity is a measure of the ability to absorb light, reflecting the degree of transparency or opacity of water, and is influenced by the amount of suspended matter present in the water column (CATIANIS et al. 2020). The average water turbidity in the studied bodies in 2019–2022 varied from 5.15 ±4.28–77.92 ±88.87 NTU (Figure 4). The results illustrate that the habitat area has the lowest turbidity in the water because it is less affected by waste sources. In addition, water environments with low pH and high Al^{3+} are hydrolyzed into $Al(OH)_3$, absorbing suspended solids in the water and dragging them to the bottom. Meanwhile, the peripheral canal area has the highest turbidity in the water, which may be affected by boat activities and other waste sources in the study area. The results also show that the turbidity in the water tended to decrease in 2022 compared to 2021. Compared with the studied water bodies at Lung Ngoc Hoang Nature Reserve (20.8–72.5 NTU), the turbidity in the water in the present study tended to be higher. High turbidity could be attributed to the high TSS concentration in the water (HONG and GIAO 2021). According to CATIANIS et al. (2020), high turbidity, i.e., the presence of organic pollution, other wastes, or runoff with high suspended matter content, reduces water visibility and affects aquatic biodiversity. Aquatic animals are only indirectly affected by the processes caused by high turbidity, such as decreased dissolved oxygen and the penetration of sunlight into the water (CATIANIS et al. 2020).

The study results showed that the average concentration of TSS in the studied water bodies from 2019–2022 ranged from 10.83 ±8.50–22.80 ±30.84 mg/L. It was found that there was no difference between TSS concentrations in water bodies during the monitoring period ($p > 0.05$). However, there was a statistically significant difference in the same water body over the years of monitoring ($p < 0.05$). TSS concentrations in aquatic habitats, rice fields, infield canals, and peripheral canals between 2019 and 2022 varied from 3.50–22.73 mg/L, 0–25.11 mg/L, 0–57.89 mg/L, and 10.3–25.53 mg/L, respectively. TSS in water tended to decrease gradually from 2021 to the present study time, similar to the previous study of LUU et al. (2020). The values of TSS in infield canals exceeded the allowable limit of QCVN 08-MT:2015/BTNMT, column A1 in 2021 (MONRE 2015). Meanwhile, the habitat area has the lowest TSS content, where there is less human influence and poor flow. Comparing the current water bodies with water bodies that are heavily affected by boat activities, domestic waste, cultivation, animal husbandry, and industry, the TSS content in the water in the Phu My protected area – habitat was much smaller (WAKAWA et al. 2010, GIAO et al. 2020, KY et al. 2020). High TSS content in water reduces the ability to transmit light into the water, thereby affecting the photosynthesis process of aquatic plants, causing depletion of dissolved oxygen in water and affecting aquatic life (HONG and GIAO 2021).

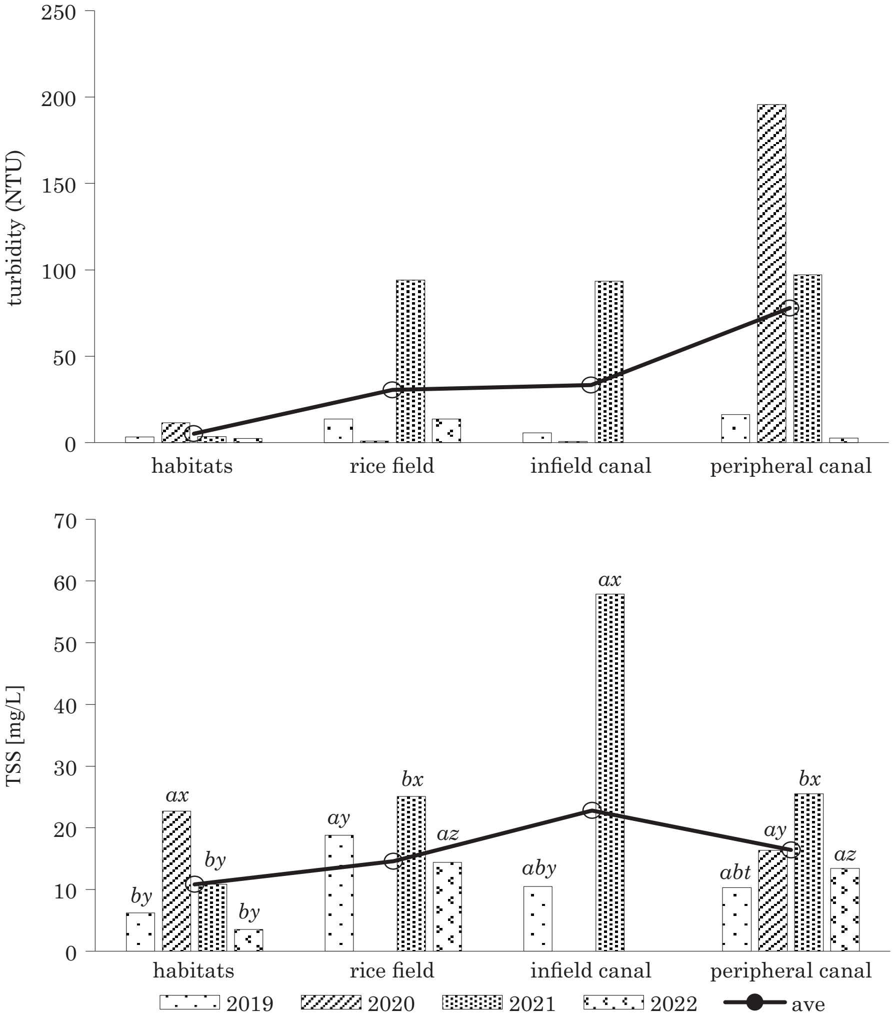


Fig. 4. Variation of turbidity and TSS in the period of 2019–2022 in the conservation area
Explanations: *a, b* are statistically significant differences between water bodies in the same monitoring year *(p* < 0.05*) and x, y, z, t* are significantly different between monitoring years in the water bodies *(p* < 0.05*)*

Dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD). The dissolved oxygen (DO) of natural water depends on temperature, surface turbulence, surface area exposed to the atmosphere, atmospheric pressure and the amount of oxygen in the surrounding air (NDUKA et al. 2008). DO concentrations in water bodies of the conservation area in 2019–2022 ranged from 3.74 ±1.14–5.71 ±1.11 mg/L (Figure 5).

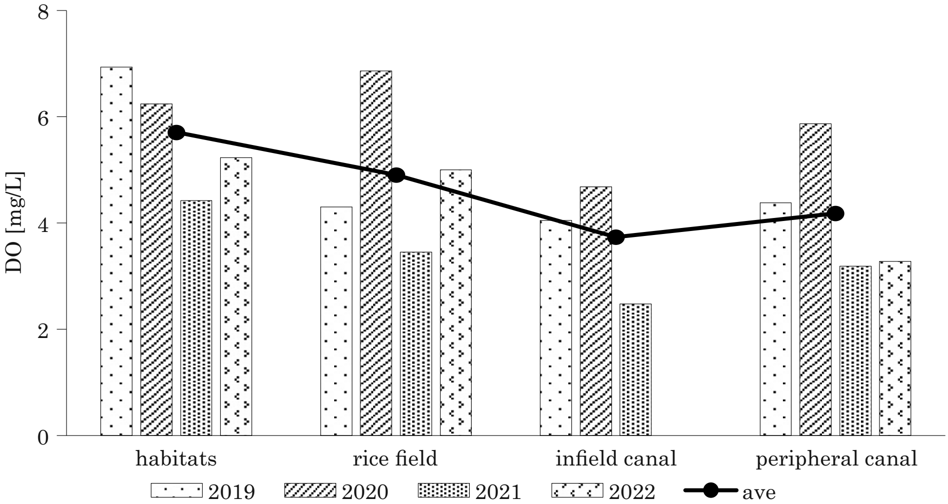


Fig. 5 Variation of DO in the period of 2019–2022 in the conservation area

DO in the infield canal (2021) and habitats (2019) were lowest and highest, with 2.48–4.68 mg/L and 4.42–6.94 mg/L, respectively. The data on rice fields and peripheral canals were 3.54–6.86 mg/L and 3.19–5.87 mg/L, in their respective order. DO tended to decrease gradually between 2019 and 2021 and increase in 2022. However, the DO content in the water in the study area does not meet the limit value of column A1 of QCVN 08-MT:2015/BTNMT, which is unsuitable for the water supply and conservation of aquatic plants and animals. The flow is poor at the main monitoring sites, and the water exchange capacity is limited, leading to low oxygen in the water. In some other water bodies, the DO content is low in water due to several reasons, such as highly suspended solids, receiving a large amount of wastewater with only partial or no treatment, and the decomposition of organic matter (WAKAWA et al. 2010, HA et al. 2016, TRUC et al. 2019). According to CATIANIS et al. (2020), DO concentrations below 5 mg/L can adversely affect biotic communities' function and survival, and below 2 mg/L can lead to fish death. According to NDUKA et al. (2008), DO concentration of 3 mg/L can interfere with fish populations through slow hatching of eggs and reduction in embryo size and viability.

The average BOD and COD concentrations in the water in the study area during the period of 2019–2022 ranged from 1.99 ±0.97–3.43 ±1.95 mg/L and 18.77 ±18.33–24.59 ±30.62 mg/L (Figure 6).

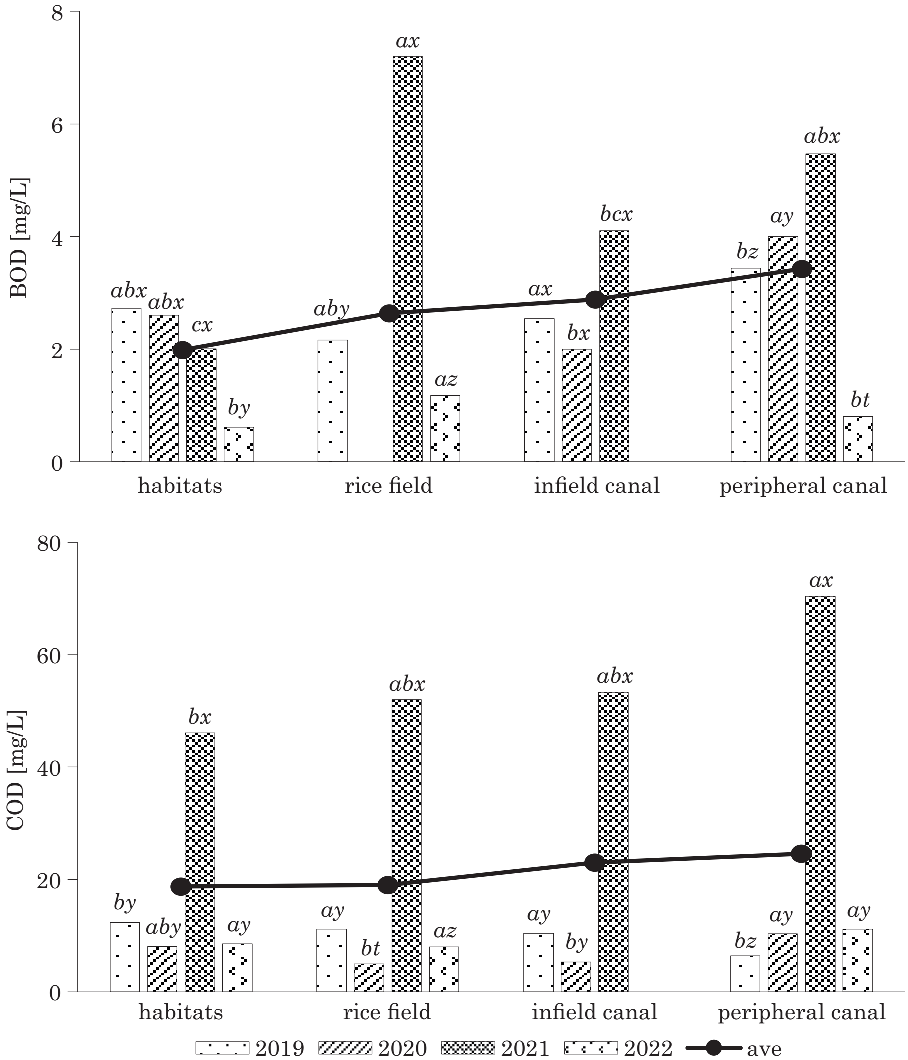
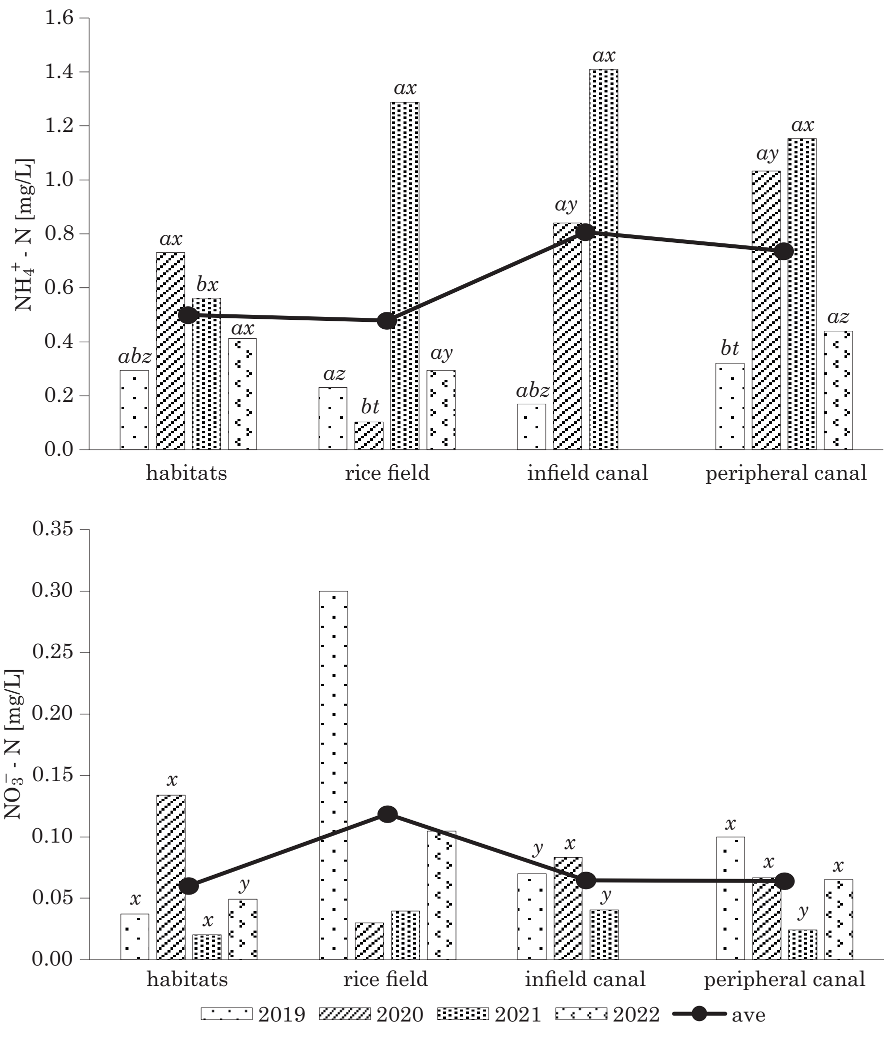


Fig. 6. Variation of BOD and COD in the period of 2019–2022 in the conservation area
Explanations: *a, b* are statistically significant differences between water bodies in the same monitoring year ($p < 0.05$) *and x, y, z, t* are significantly different between monitoring years in the water bodies ($p < 0.05$)

Statistical analysis found that the BOD and COD concentrations between water bodies in the same year were not statistically significant ($p > 0.05$). The evolution of BOD content in each habitat, rice field, infield canal, and peripheral canal in the period of 2019–2022 fluctuated in the range of 0.61–2.73 mg/L, 0–7.20 mg/L, 2–4.10 mg/L, and 0.80–5.47 mg/L, respectively. For COD content, it fluctuated from 8.07–46.11 mg/L, 5–52.02 mg/L, 5.33–53.33 mg/L, and 6.40–70.41 mg/L, respectively, for habitats, rice fields, and infield canals and external canals. This result shows that the aquatic habitats have the lowest BOD and COD concentrations among the four surveyed water bodies and are suitable for conserving aquatic plants and animals. Statistical analysis results show that the organic matter content in the rice fields and the peripheral canals fluctuated significantly over the years ($p < 0.05$). Besides, the results also show that the BOD concentration decreased at present compared to the previous year (in 2021). At the same time, the COD content tended to fall in 2019–2020 and increase sharply, exceeding the allowable limit of QCVN 08-MT:2015/BTNMT, Column A1 in 2021 and decreasing in 2022. In general, the concentration of BOD in the conservation area is still within the allowable limit of QCVN 08-MT:2015/BTNMT, Column A1. Nevertheless, the COD concentration in the water bodies of the study area was relatively high and exceeded the permissible limit. From the above analysis, it is proved that the aquatic habitats in the NR have the best environmental quality in terms of organic pollutants, with both BOD and COD content lower than the remaining water bodies.

Nutrients (NH_4^+-N, NO_3^--N, PO_4^{3-}-P, TN). The concentration of ammonium in water in water bodies ranges from 0.48 ±0.55–0.81±0.65 mg/L from 2019 to 2022, reaching the highest value in the infield canal water bodies (in 2021) and the lowest in the rice fields (in 2020). The content of NH_4^+-N in each habitat, rice field, infield, and peripheral canals with values of 0.29–0.73 mg/L, 0.10–1.29 mg/L, 0.17–1.41 mg/L and 0.17–1.41 mg/L, 0.32–1.15 mg/L, respectively (Figure 7). NH_4^+-N content increased gradually from 2019–2021, followed by a decrease in 2022. However, the average NH_4^+-N concentration in water bodies has exceeded the allowable limit of QCVN 08-MT:2015/BTNMT (column A1) for aquatic flora and fauna conservation and domestic water supply. Based on the statistical analysis results, there was no statistically significant difference in the concentration of NH_4^+-N in water bodies in the same year ($p > 0.05$). In contrast, the fluctuations over the surveyed period in each water body were found to be a statistical difference at a 95% significance level ($p > 0.05$). According to Boyd and Green (2002) classified the level of eutrophication in freshwater systems for nitrogen concentration from 0.18–0.43 mg/L. Thereby, there was a sign of eutrophication in the study area. The nitrate content in water in the conservation is very low, still within the allowable limit of QCVN 08-MT: 2015/BTNMT. The concentration of NO_3^--N in water bodies fluctuated from 0.06 ±0.03–0.12 ±0.13 mg/L in 2019–2022. Statistical analysis showed that the NO_3^--N concentration in the same water body was not a statistically significant difference (p > 0.05). NO_3^--N has gradually increased from 2021 to the current study time (in 2022).



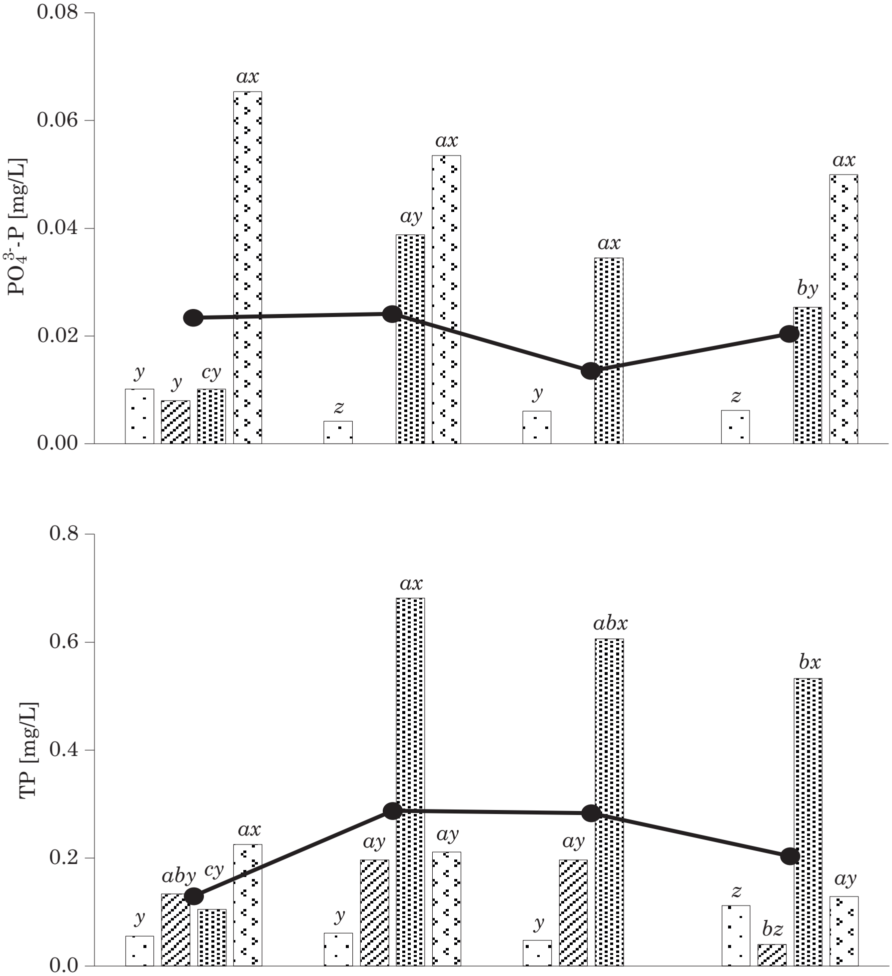


Fig. 7. Variation of nutrients in the period of 2019–2022 in the conservation area
Explanations: *a, b* are statistically significant differences between water bodies in the same monitoring year *($p < 0.05$) and x, y, z, t* are significantly different between monitoring years in the water bodies *($p < 0.05$)*

Phosphorus has a great role in biological metabolism, is an essential nutrient element, and plays a crucial role in photosynthesis and other processes in plants and algae (HENEASH et al. 2021). However, the presence of phosphorus in the form of PO_4^{3-}-P in the aquatic environment is the main cause of eutrophication (NDUKA et al. 2008). The research results show that the concentration of PO_4^{3-}-P in the water bodies of the conservation area has a relatively low value and was still within the allowable limit of QCVN 08-MT:2015/BTNMT. The average TP concentration ranged from

0.13 ±0.07–0.29 ±0.27 mg/L and was high in the canals and rice fields. There are signs of a rise from 2021 to 2022. Specifically, the concentration of PO_4^{3-}-P in water bodies in 2019–2022 ranges from 0.01 ±0.02–0.02 ±0.03 mg/L. The statistical analysis results show that PO_4^{3-}-P concentration between water bodies significantly differed ($p < 0.05$).

Iron (Fe^{2+}) and aluminum (Al^{3+}). The concentrations of Al^{3+} in the studied water bodies have an average value ranging from 2.58 ±2.15–145.04 ±213.93 mg/L (Figure 8). Al^{3+} concentration in the habitat water was the highest among the studied water bodies. The results also showed that the Al^{3+} concentration in each water body fluctuated over the years with statistically significant differences ($p < 0.05$). The results of the study in the period 2019–2021 were similar to the previous record of NI (2018), which reported that the Al^{3+} content ranged from 2.85–21.25 mg/L. However, the significant increasing trend of Al^{3+} content in 2022 could be explained by the dredging of deep alum soil to create channels/dykes in the conservation area, making a closed water body leading to a potential alum soil layer leaching into canals in the conversation area's habitats. The average concentration of Fe^{2+} in water bodies ranges from 2.17 ±2.74 to 5.25 ±3.69 mg/L. In aquatic habitats, rice fields, intra-field canals, and peripheral canals, Fe^{2+} concentration fluctuates in the range of 2.34–4.58 mg/L, 0.17–6.12 mg/L, 1.52–8.89 mg/L, 0.07–6.46 mg/L, respectively. Fe^{2+} content in water tends to be highly concentrated in the peripheral canal, increasing gradually from 2019–2021 and decreasing in 2022. Fe^{2+} concentration in the study area is high, mainly because acidic soil releases Fe^{2+} into the water. The analysis results also show that the Fe^{2+} concentration fluctuates in each water body over the years with statistically significant differences ($p < 0.05$). According to MINH et al. (2006), potential acid sulfate soils will have high FeS_2 content, so when FeS_2 is oxidized, Fe^{2+} is released. According to DONG et al. (2015), water contaminated with iron is water with low pH, consistent with the pH results in the study area, then the water will have a fishy smell and much yellow dirt. The Fe^{2+} content in the study is thought to have no significant effect on growth and development in the habitats in the reserve, but it is not suitable for rice cultivation (GIAO et al. 2020). The analysis results illustrate that water quality in the study area tended to be contaminated with aluminum.

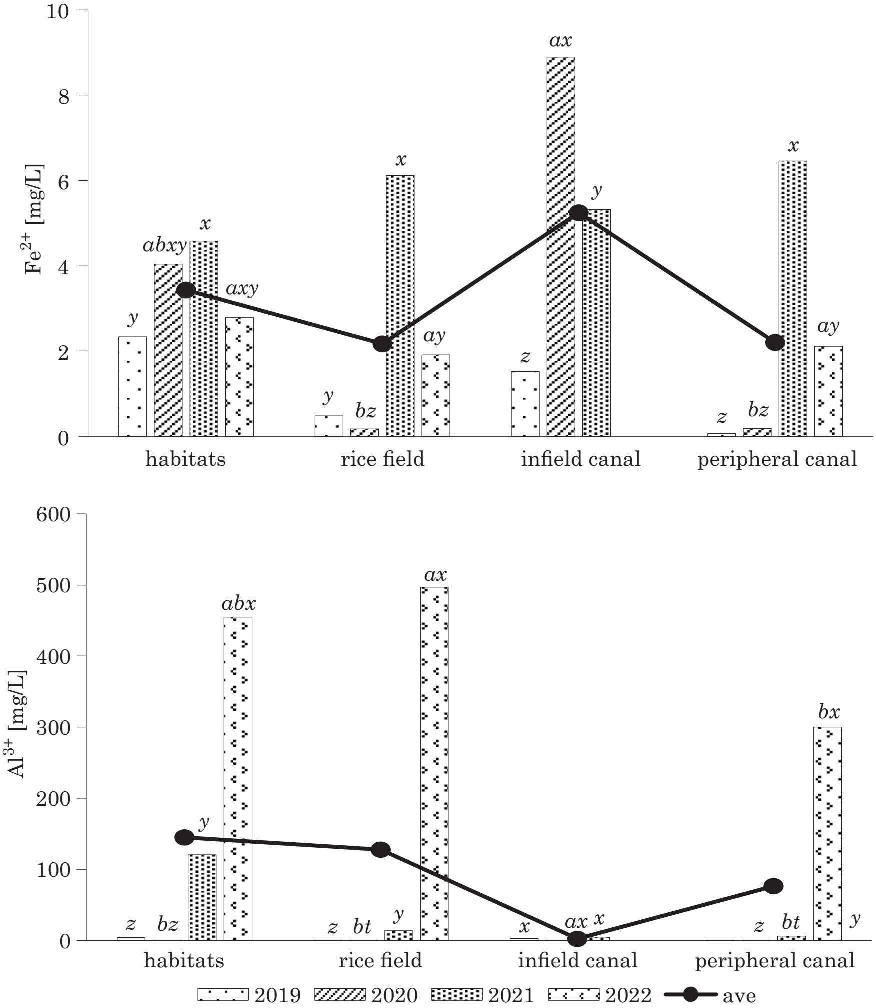


Fig. 8 Variation of iron and aluminum in the period of 2019–2022 in the conservation area
Explanations: *a, b* are statistically significant differences between water bodies in the same monitoring year *(p* < 0.05*)*, and *x, y, z, t* are significantly different between monitoring years in the water bodies *(p* < 0.05*)*

Correlation among surface water parameters in the conservation area

Pearson correlation analysis was performed from data from 14 parameters in the water bodies, as detailed in Table 1. Table 1 shows that pH was closely correlated with most parameters, with negatively correlated with EC (-0.717), salinity (-0.639), DO (-0.354), and positively correlated with turbidity (0.630), TSS (0.673), COD (0.592), NH_4^+-N (0.519), TP (0.626). EC was strongly correlated with salinity (0.934), consistent with research results.

Table 1

Correlation of surface water quality variables

Parameters	pH	EC	Sal	Turb	TSS	DO	BOD	COD	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	TP	Fe^{2+}	Al^{3+}
pH	**1**	–	–	–	–	–	–	–	–	–	–	–	–	–
EC	-0.717**	**1**	–	–	–	–	–	–	–	–	–	–	–	–
Sal	**-0.639****	**0.934****	**1**	–	–	–	–	–	–	–	–	–	–	–
Turb	**0.630****	**-0.495****	**-0.443****	**1**	–	–	–	–	–	–	–	–	–	–
TSS	**0.673****	**-0.514****	**-0.475****	**0.508****	**1**	–	–	–	–	–	–	–	–	–
DO	-0.354*	**0.414****	**0.354***	-0.212	-0.283	**1**	–	–	–	–	–	–	–	–
BOD	0.217	-0.059	-0.045	0.128	0.087	-0.186	**1**	–	–	–	–	–	–	–
COD	**0.592****	**-0.444****	**-0.380***	**0.356***	**0.484****	**-0.319***	-0.143	**1**	–	–	–	–	–	–
NH_4^+-N	**0.519****	-0.124	-0.096	**0.557****	**0.512****	-0.188	0.149	**0.454****	**1**	–	–	–	–	–
NO_3^--N	0.084	-0.248	-0.274	0.058	0.271	-0.101	0.076	-0.219	0.019	**1**	–	–	–	–
PO_4^{3-}-P	0.145	0.035	0.118	0.036	0.035	-0.229	**0.775****	-0.149	0.139	0.044	**1**	–	–	–
TP	**0.627****	**-0.414****	-0.300	**0.492****	**0.571****	**-0.322***	**0.349***	**0.428****	**0.535****	-0.006	**0.505****	**1**	–	–
Fe^{2+}	0.094	0.227	0.291	0.056	0.097	-0.138	0.019	**0.387***	**0.427****	-0.217	0.021	0.286	**1**	–
Al^{3+}	-0.082	0.159	0.205	-0.206	-0.209	-0.046	**0.637****	-0.249	-0.053	-0.081	**0.756****	0.065	0.024	**1**

Explanations: ** correlation is significant at $p < 0.01$ level; * the correlation is significant at the level $p < 0.05$

Meanwhile, both EC and salinity showed a weak negative correlation with turbidity and COD. EC also appeared to have a weak negative correlation with TP (-0.414). For turbidity and TSS, there was a positive correlation with COD (0.356, 0.484), NH_4^+-N (0.557, 0.512), and TP (0.492, 0.571). In addition,, turbidity also has a positive correlation with TSS (0.508). TSS appeared to have a weak and moderate positive correlation with organic pollutants (COD) and nutrient pollutants (NH_4^+-N, TP). DO is negatively correlated with COD and TP, with correlation coefficients of -0.319 and -0.322, respectively. Decomposing organic compounds and nutrients has contributed to reducing dissolved oxygen in water. The research results also show that organic pollutants and nutrients positively correlate. It is shown that they have a common origin, which could be derived from the discharge of domestic wastewater, agricultural runoff, and the use of fertilizers in farming in the study area. In addition, COD and NH_4^+-N have a weak correlation with Fe, respectively; BOD and PO_4^{3-}-P were strongly correlated with the formation of Al^{3+} in water. The correlation coefficient between the study area's surface water environment variables is significant (p < 0.01). Besides the factors analyzed in the study, a previous report by TRAN et al. (2019) indicated that threats from climate change, such as drought, hydrological change, and inundation due to sea level rise, could significantly affect protected areas because of the low adaptive capacity of conservation areas.

Clustering surface water quality in the study area

Cluster analysis (CA) was performed from the data on eight monitoring locations in 2022 in order to find monitoring locations with similar characteristics, which gathered into a group, while sites with different physical and chemical characteristics will form separate groups.

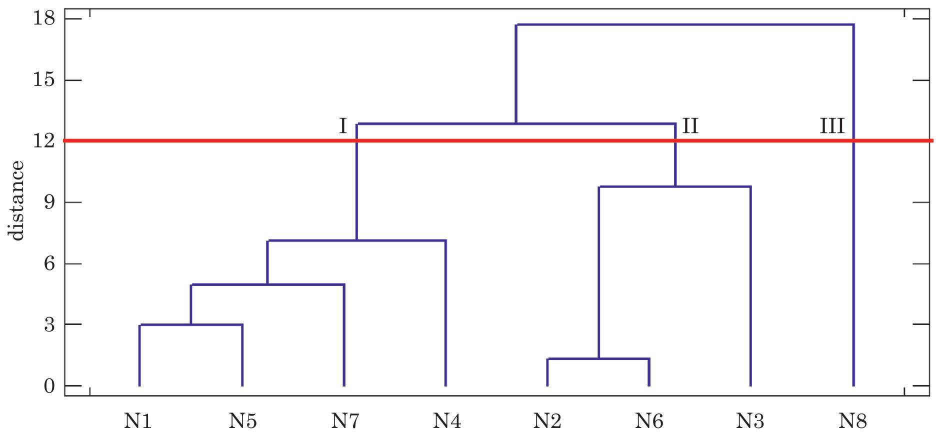


Fig. 9. Clustering surface water quality in the study area

The cluster analysis results formed three groups of surface water quality (Figure 9). Group I was established by four locations (i.e., N1, N4, N5, and N7), where peripheral canals, Melaleuca – Eleocharis, Melaleuca, and rice fields are located. Similarly, N2, N3, and N6 have similar characteristics and were classified into Group II, while Group III only included one location. Group II indicated that all locations are located in conservation areas' habitats, namely Melaleuca – Eleocharis, Lepironia – Eleocharis, and Lepironia. On the other hand, N8 was also found in the Melalecu habitat; however, this location is planned for the Melaleuca plantation, thereby, it could be affected by soil disturbance, leading to water quality characterized by high dissolved ions and low turbidity. The remaining locations have high levels of total suspended solids and organic matter. Based on the analysis results, it could be seen that the monitoring network of water quality in the conservation area was suitable for the status and activities of the conservation since the monitoring was carried out.

Conclusion

The research results show that the surface water environment at Phu My Species-Habitat reserve is acidic, and iron and aluminum are high in the water. Nutrient pollutants (NO_3^--N and PO_4^{3-}-P) are relatively low. Organic pollutants (BOD and COD) decreased in 2022 compared to 2021. In general, organic and nutrient pollution in the reserve is not high and has not significantly affected the life of aquatic plants and animals. Surface water quality in the reserve is being well managed for the development of Lepironia and Eleocharis. Pearson analysis results show that organic pollutants and nutrients in water have a positive correlation with each other, having the same origin from the discharge of domestic wastewater agricultural runoff in the study area. CA analysis results demonstrate that the monitoring system in the conservation was suitable for evaluating water quality. It could be applied to monitoring water quality annually in the future.

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