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Camptostemon philippinensis photo by dfjtees

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Figures and Tables of maximum of three pages should be clearly presented. Title of a picture is written down below the picture, while title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; chart is preferred to use black and white images. Author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned source. **There is no appendix**, all data or data analysis are incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

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Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

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Community structure of phytoplankton in the surface and thermocline layers of Sangihe and Talaud waters, Indonesia

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Manuscript received: 9 July 2019. Revision accepted: 15 August 2019.

Abstract. *Sriwijayanti LA, Djumanto, Setiawan RY, Firdaus MR, Fitriya N, Sugeha HY. 2019. Community structure of phytoplankton in the surface and thermocline layers of Sangihe and Talaud waters, Indonesia. Bonorowo Wetlands 9: 57-64.* This study aimed to determine the species dominance and distribution and community structure of phytoplankton in the surface and thermocline layers of Sangihe and Talaud waters Indonesia. Phytoplankton samples were collected at the Sangihe-Talaud waters in October 2018 at 14 research stations. Water samples were collected at 5 m (surface) and thermoclines layers using a rosette sampler equipped with a Conductivity, Temperature, and Depth (CTD) recorder. Samples were concentrated to 40 ml using hand plankton net (mesh size 20 µm), then preserved with 4% formaldehyde. Phytoplankton species were identified using a guidebook based on morphological character traits. The cell count of each plankton species was calculated using a Sedgwick rafter counting cell chamber. The result showed 4 classes of phytoplankton (Bacillariophyceae, Dinophyceae, Cyanophyceae, and Raphydophyceae), consisting of 59 species in the surface 56 species in the thermocline, respectively. The abundance of phytoplankton at the surface ranged from 77,333-4,024,000 cell m^{-3}, meanwhile in the thermocline layer, 8,000-542,222 cell m^{-3}. The average phytoplankton diversity of the surface was 0.82, and the thermocline was 1.71. The surface layer was dominated by *Leptocylindrus danicus* (8.92 x 106 cell m^{-3}), *Trichodesmium erythareum* (5.83 x 106 cell m^{-3}), and *Detonula converfacea* (0.62 x 106 cell m^{-3}). The thermocline layer was dominated by *Chaetoceros affinis* (2.74 x 105 cell m^{-3}), *Thalassionema nitzchioides* (2.21 x 105 cell m^{-3}), and *Chaertoceros dichaeta* (1.38 x 105 cell m^{-3}). The low phytoplankton abundance found at stations 12 and 13 was caused by higher salinity concentrations. The highest phytoplankton abundance was found in the stations with warmer temperatures, both on the surface and in the thermocline. The shallow depth thermocline layer (75-100 m) is more abundant than the deeper thermocline layer (110-150 m). The temperature was the environmental parameter that had the greatest influence on the abundance and species of phytoplankton; the phytoplankton in the surface layer reached 10 times more abundant than the thermocline layer.

Keywords: Phytoplankton, surface, thermocline, tropical

INTRODUCTION

Plankton is a group of microscopic organisms found in almost all types of waters, moving passively following the flow; their biomass in marine waters reaches 98% of all micro-sized organisms (Sardet 2015). Phytoplankton is a group of plankton that can photosynthesize and contribute to almost half of the total global net primary productivity (Falkowski et al. 1998). As a primary producer, phytoplankton is a food source for all populations in the sea (Lagus et al., 2004; Sardet, 2015; Rowe et al., 2017). The first consumer of phytoplankton is zooplankton, a food source of marine biotas such as fish, shrimp, lobsters, crabs, and various types of small fish. Many studies show that phytoplankton has a positive correlation between high commercial fish catches such as mackerel (Tangke 2012), sardinella (Putra et al. 2012), and tuna (Tangke et al. 2015; Tangke et al. 2016). In addition, the four types of high commercial fish mostly live in the thermocline layer. However, the existents of phytoplankton tend to follow the movement towards water currents. It is also significantly affected by physical and chemical changes in the waters. Depth, temperature, and salinity are crucial parameters determining the phytoplankton community structure horizontally and vertically (Sardet 2015).

The water column vertically has a different density gradient depending on the temperature and depth. The temperature will decrease to seawater depth. Otherwise, water pressure will increase. The temperature will drop dramatically at a certain depth, called the thermocline layer. In addition to temperature, salinity also has a similar pattern, which will increase dramatically at a certain depth, and it is referred to as a halocline layer. The thermocline and halocline layers create unique conditions that make phytoplankton adaptable to survive. Phytoplankton communities make different adaptations so that there are variations in community structure between water columns based on their abilities and characteristics of life.

Sangihe Talaud waters directly adjacent to the Mindanao Islands (southern Philippines) have water masses affected by North Pacific waters (Gordon 2005). This water mass will flow through the thermocline layer (Koch-Larrouy et al. 2007) to provide different water conditions with the surface layer. Indirectly it will form the structure

of the phytoplankton community that lives in it. Various studies on plankton dynamics have been carried out in Indonesian waters. However, research on the phytoplankton community structure in Indonesia's surface and thermocline layers is rarely reported, especially in the Sangihe Talaud waters. Phytoplankton is the basis of the food chain. Research on the abundance and species of phytoplankton in the surface and thermocline layers is very important, especially in waters that become fishing ground for fishes with high economic value. Therefore, this study aims to determine the phytoplankton community structure in the surface layer and thermocline in the Sangihe Talaud Sea Waters.

MATERIALS AND METHODS

Study area

The research was conducted at Sangihe Talaud Waters in October 2018. Sampling was carried out at 14 research stations located on the northeastern side of Sulawesi Island (2 ° 4 '13 "- 4 ° 44 '22" N) and (125 ° 9 '28 " - 125 ° 56 '57 "E) (Figure 1). Sampling was done using the Baruna Jaya VIII Research Vessel belonging to the Indonesian Institute of Sciences (P2O LIPI).

Procedures

The temperature, salinity, and depth parameters were measured using the SBE 911-Plus CTD (Conductivity Temperature Depth) with Carousell Water Sampler Sensor. This tool was equipped with 12 rosette sampler bottles with a capacity of 10 liters, and it was used to take water samples as phytoplankton samples at surface depth (5 m) and thermoclines layers. The phytoplankton samples were filtered using hand plankton net mesh size 20 µm. The collected filtrate was transferred to a 40 ml sample bottle and preserved using 1% Lugol. Phytoplankton enumeration was done using Sedwick-Rafter Counting Cell under a microscope with 100x magnification. Phytoplankton identification was carried out morphologically by referring to the book Yamaji (1976), Shirota (1996), and Omura et al. (2012).

Data analysis

The results of enumeration and identification of phytoplankton were then used to analyze phytoplankton communities based on their abundance. Phytoplankton abundance was calculated using a formula according to Perry (2003), which was modified by Huliselan et al. (2006):

$$D = (Nf*Vp)/v$$

D = plankton abundance (ind / m³)
Nf = number of cells per 1 ml
Vp = dilution volume (ml)
V = volume of filtered water (m³)

The diversity of phytoplankton is determined by the following equation (Spellerberg and Fedor 2003):

$$H' = - \sum P_i \ln P_i$$

H' = diversity index
Pi = Proportion of species = Pi = ni / N
Ni = number of individuals of a species
N = Total number of individuals of all species

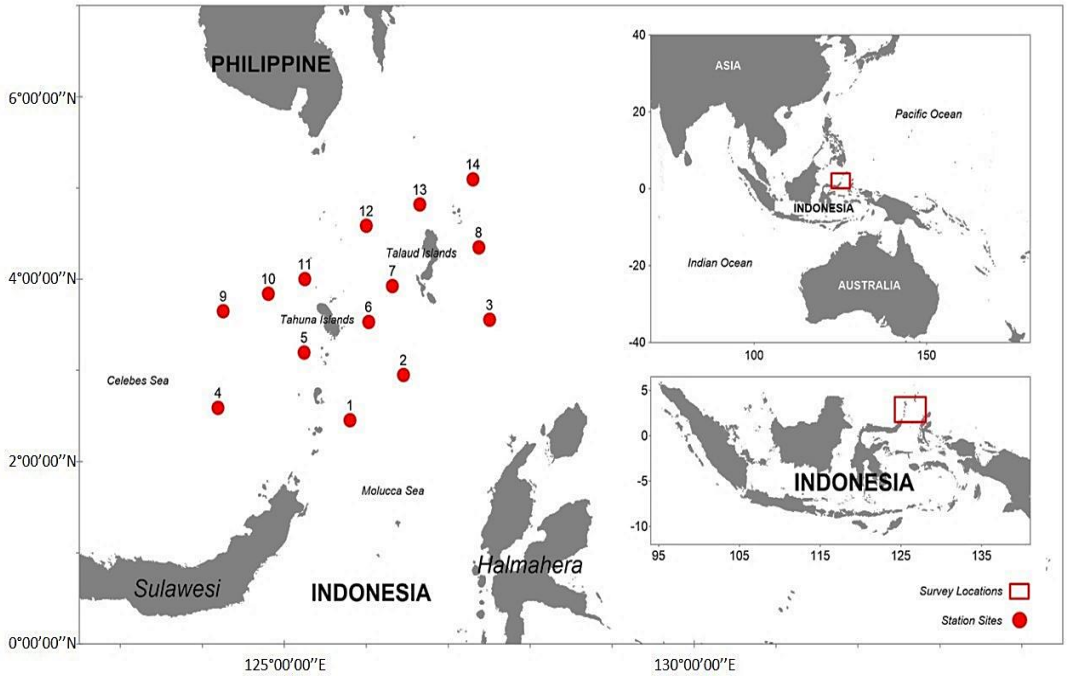

Figure 1. The map showing research station (indicated number 1 to 14) in the Sangihe-Talaud Warters, North Sulawesi, Indonesia

The diversity index was categorized based on Krebs (1989): (i) H'<1.0: small diversity (high ecological pressure), (ii) 1.0 <H'<3.322: medium diversity (productivity is quite good, the ecosystem is quite balanced, pressure is ecologically balanced), (iii) H'> 3.322: high diversity (very high productivity).

The relationship of water quality with the abundance of phytoplankton at each station was mapped in the form of contours using Surfer 9.1.352.

RESULTS AND DISCUSSION

Physical and chemical parameters

The values of physical and chemical parameters of waters such as temperature and salinity in Sangihe Talaud waters were taken up to a depth of 600 m to clearly describe the stratification profile in the thermocline layer and the layer below the thermocline (Steele and Thorpe 2009). Vertical profiles of temperature and salinity in 14 stations are shown in Figure 2.

Figure 2 shows that the water layer increased deeper, causing salinity to increase, but the temperature decreased. The temperature and salinity of the surface were 29.23-30.24°C and 33.55 - 34.31 ‰, while on the thermocline layers were 14.83-27.04°C and 34.5-35.08 ‰, respectively. The average temperature and salinity in the surface was 29.64 ± 0.29°C and 34.07 ± 0.22 ‰, while in the thermocline layers were 19.95 ± 02.23°C and 34.80 ± 0.12 ‰, respectively. The temperature on the surface, 30.24 °C, dropped drastically until the thermocline layer reached 14°C, while the salinity didn't show a significant increase. The average depth in the thermocline layer of the Sangihe-Talaud waters was 130 m.

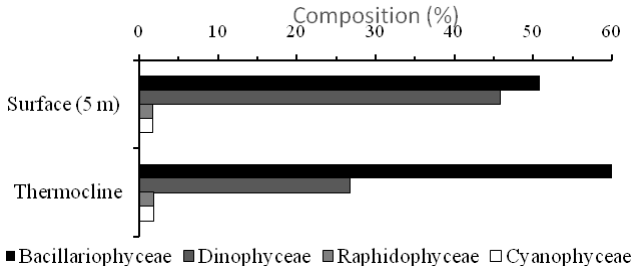

Figure 3. Composition of the number of phytoplankton species based on the class composition of surface and thermoclines layers.

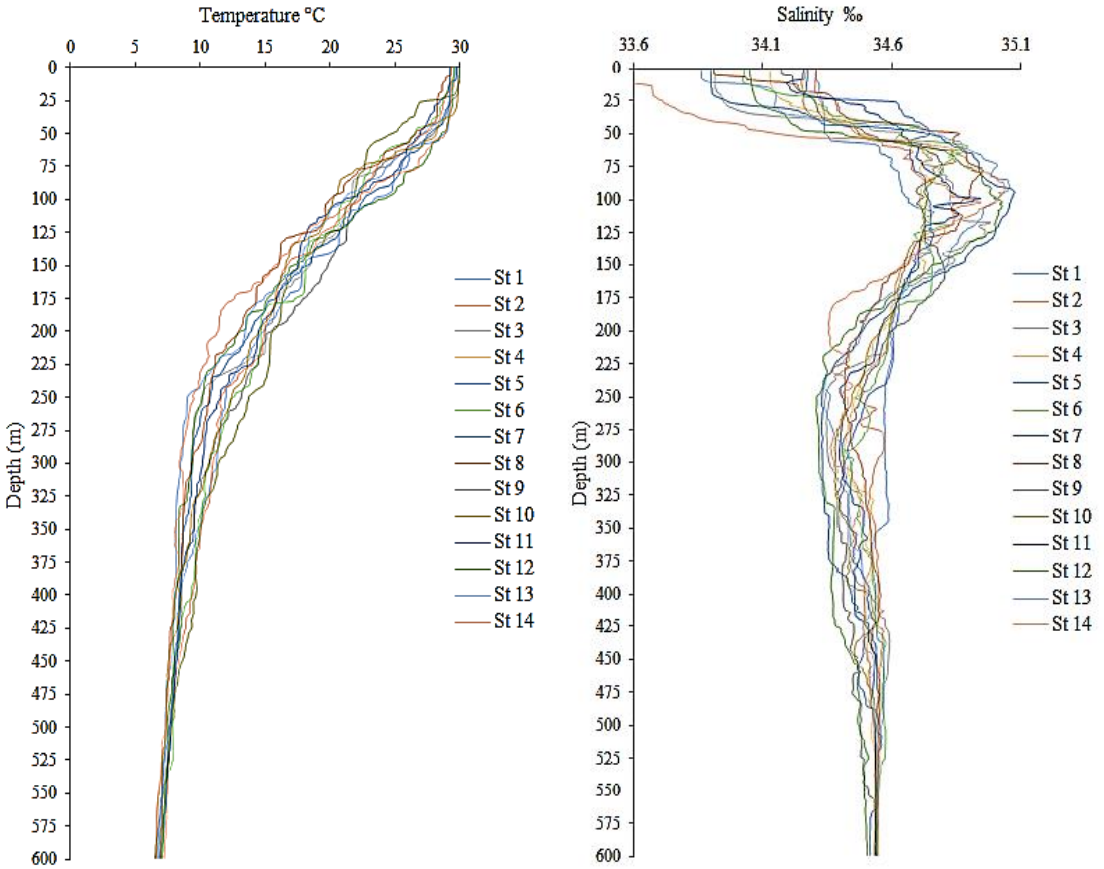

Figure 2. Vertical profile of temperature and salinity in the Sangihe Talaud waters, Indonesia

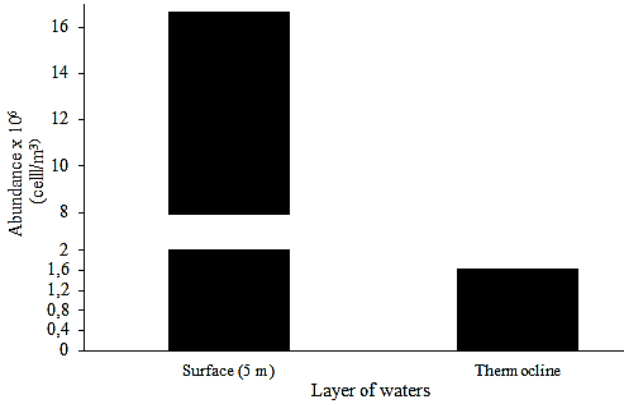

Figure 4. The abundance of phytoplankton on the surface and thermocline layers in the Sangihe-Talaud waters

Abundance and diversity of phytoplankton

Phytoplankton identification consisted of 4 classes, namely Bacillariophyceae, Dinophyceae, Cyanophyceae, and Raphydophyceae, with a total of 59 species on the surface 56 species in the thermocline layers. The number of species based on the phytoplankton class is shown in Figure 3.

The surface was dominated by Bacillariophyceae 50.85%, and Dinophyceae 45.76%, and the remaining was from Cyanophyceae and Raphidophyceaen, each 1.69%. The dominance numbers of the Bacillariophyceae in the thermocline layer were found more significant than the surface at sum 69.64%, the remaining from the Dinophyceae 26.79%, and from the Cyanophyceae and Raphidophyceae each 1.79%. The total abundance of phytoplankton in the surface layer and the thermocline shows a significantly different value, namely the surface layer 10 times greater than the thermocline. This condition is presented in Figure 4.

The phytoplankton abundance in the surface layer ranged 77,333 - 4,024,000 cell m-3; meanwhile, the thermocline layer ranged from 8,000 to 542,222 cell m^{-3}. Phytoplankton dominant and abundant species in surface waters differed from the thermocline layer. The surface was dominated by *Leptocylindrus danicus, Trichodesmium erythareum,* and *Detonula converfacea,* while the thermocline layer was dominated by *Chaetoceros affinis, Thalassionema nitzchioides,* and *Chaertoceros dichaeta* (Figure 5).

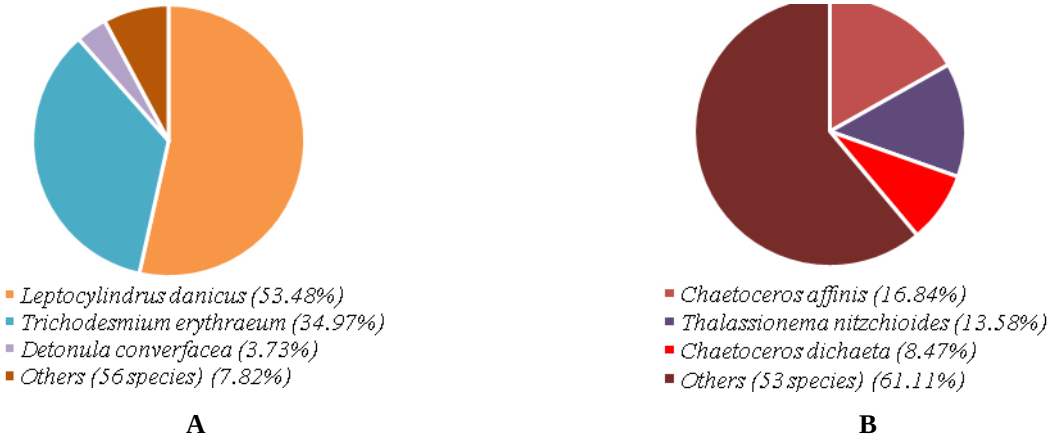

Figure 5. The composition of predominant phytoplankton species on the surface (A) and the thermocline layer (B)

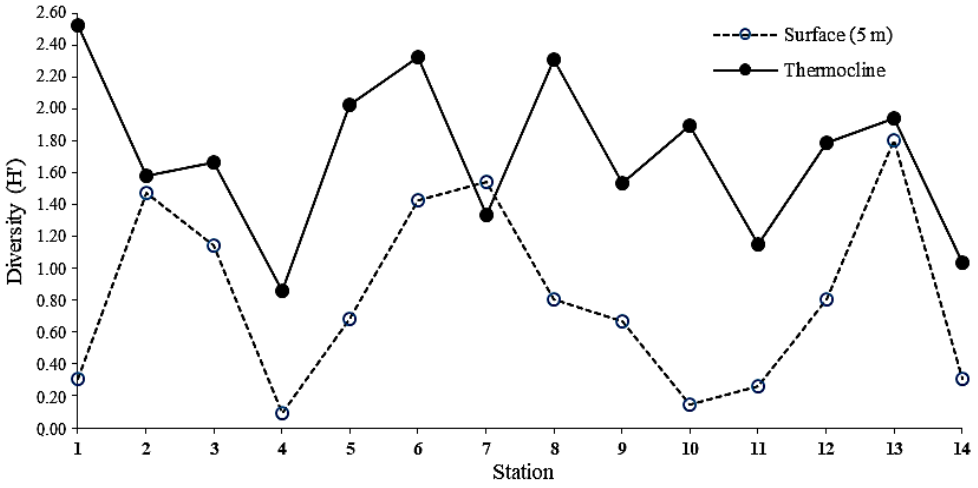

Figure 6. Diversity value (H ') at 14 stations of Sangihe-Talaud waters *(surface and thermocline)*

Among the research stations, especially on the surface, *L. danicus* has an abundance value of 8.92 x 10^6 cell m^{-3}, T. *erythareum* 5.83 x 10^6 cell m^{-3}, and *D. converfacea* 0.62 x 10^6 cell m^{-3}. The smaller value of the total abundance at the thermocline layer was *C. affinis* 2.74 x 10^5 cell m^{-3}, *T. nitzchioides* 2.21 x 10^5 cell m^{-3}, and *C .dichaeta* 1.38 x 10^5 cell m^{-3}. Although the abundance of phytoplankton was concentrated in the surface layer, it was not followed by high phytoplankton diversity values (H'). Figure 6 shows that H 'phytoplankton in the surface was lower than the thermocline layer.

The diversity of Phytoplankton (H') in the surface ranged from 0.089 to 1.807 with an average of 0.81, whereas in the thermocline layer ranged from 0.86 - 2.52 with an average of 1.71. Based on the category of diversity value, the diversity of phytoplankton in the surface layer was small; meanwhile, the thermocline layer was a medium.

Effect of environmental parameters on phytoplankton abundance between stations

The abundance of phytoplankton among stations varied because several stations had a very high abundance, while others were much lower. This is illustrated in Figure 7.

At surface waters, high phytoplankton abundance was found at stations 5, 4, 11, and 1, while low abundance was found at stations 6, 7, 13, and 2. In the thermocline layer, the highest phytoplankton abundance was found at stations 8, 1, 5, and 6, while low abundance was found at stations 14, 11, 10, and 7. Water's physical and chemical properties, such as temperature and salinity, were closely related to phytoplankton's life, indirectly affecting its distribution. This phenomenon is described as a contour pattern in Figure 8.

Layers with warm temperatures and relatively uniform salinity were found at stations 10, 4, 5, and 11 (29.64°C and 34.07 ‰). This was the reason for the high abundance of phytoplankton in the study area. Thermoclines with an average warm temperature were found at stations 8, 1, 5, and 6. The highest abundance values followed warm temperatures and low thermocline depths (75-100 m). However, stations 12 and 13 with relatively warm temperatures (21°C), high salinity, and relatively shallow depths (100 m) do not have high phytoplankton abundance.

Discussions

Oceanographic parameters observed in the Sangihe Talaud waters influenced each other. Temperature affects salinity by increasing seawater density as depth increases (Thurman 1993; Hadikusumah 2008). In addition, salinity was also related to gravity and buoyancy. When the depth increases, heavier water masses tend to sink to reach equilibrium, and less dense water will rise to the surface. The temperature profile decreases along with increasing depth due to the penetration of sunlight decreases to transfer heat to the deeper water column (Nontji 2002).

The Bacillariophyceae class dominated phytoplankton composition found in the surface or thermocline layer. Extensive distribution for the Bacillariophyceae family in the waters because of their high ability to survive to adapt to various environmental characteristics (Arinardi et al., 1996). The discovery of Bacillariophyceae, which predominates in the thermocline layer, was supported by the ownership of pigments such as fucoxanthin, chlorophyll-a, and chlorophyll-c to utilize them minimal light for photosynthesis (Rissik 2009).

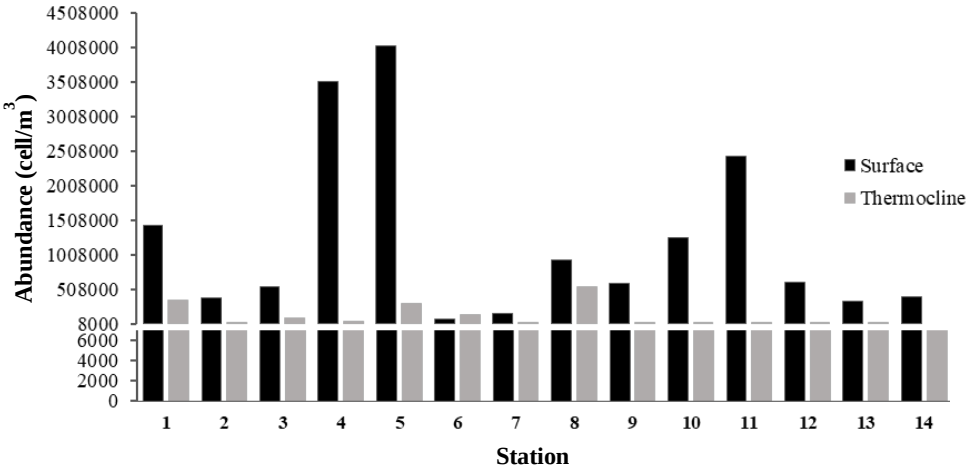

Figure 7. The abundance of total phytoplankton species at each sampling station in the surface and thermocline layer

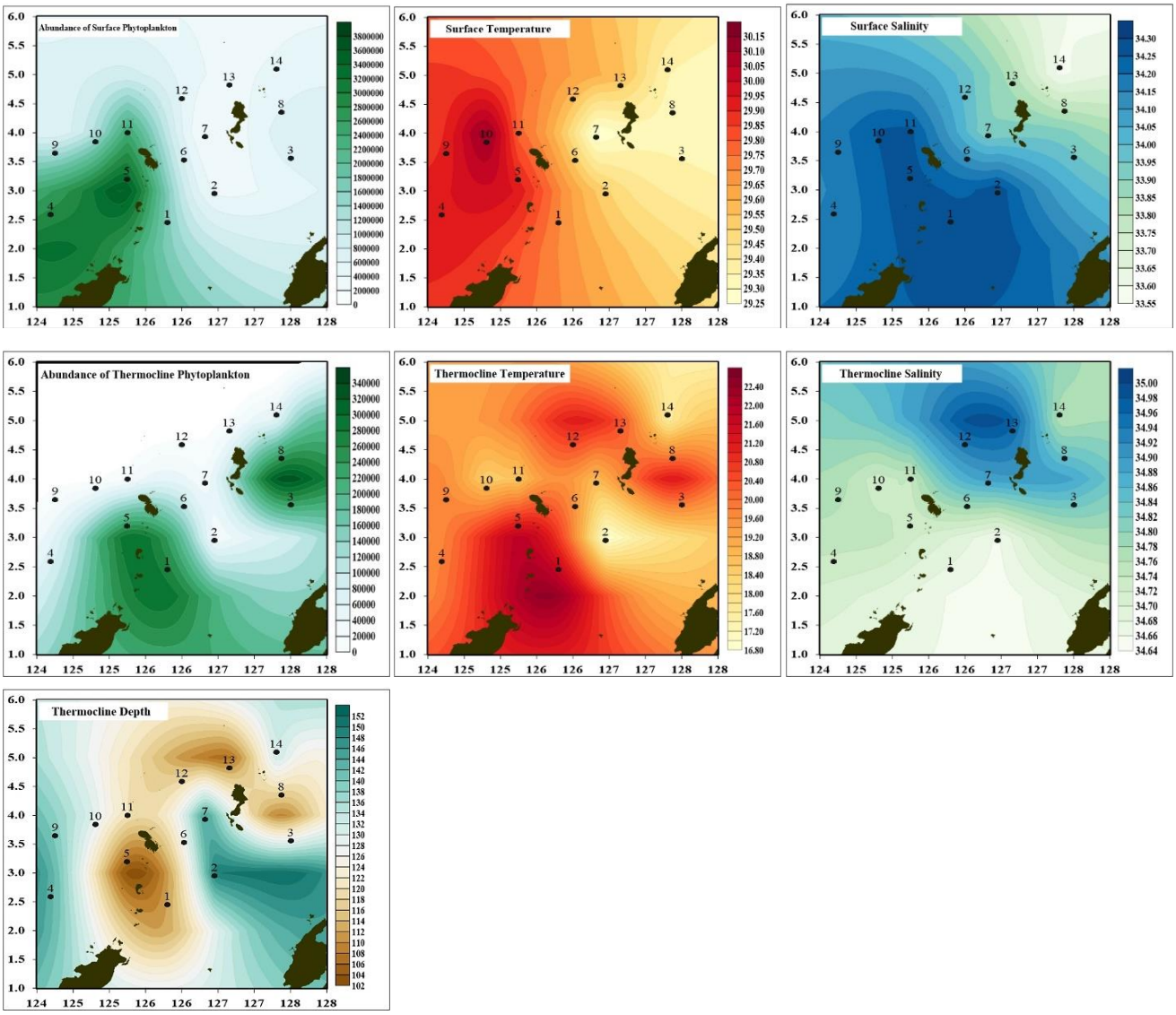

Figure 8. The contours of the relationship of phytoplankton abundance, temperature, salinity, and thermocline depth An explanation of the legend of each picture is presented on each panel

The abundance of phytoplankton in the surface layer and thermocline has a contrast difference, where abundance in the surface layer can reach 4 million cells m^{-3}. At the same time, there are no more than 1 million cells in the thermocline m^{-3}. Differences in aquatic conditions also show variations in the types of phytoplankton that live and affect abundance. The types of phytoplankton with high abundance in the surface layer were *L.danicus*, as much as 53.48%. This phenomenon is common in marine environments worldwide (Karthik 2017). It is known that the species L.*danicus* dominates 95-99% (67,000 cells) of the total abundance of phytoplankton in the Coastal Waters of Andaman and Nicobar, India (Karthik 2017). The ability to tolerate intense light and warm temperatures caused *L. danicus* to live well on the surface layer. This phenomenon was supported by the results of Penelope et al.'s (2016) study, who found that *L. danicus* grew more slowly at 18°C, while at warmer temperatures (25°C) showed good productivity.

The second dominant species in the surface layer was *T. erythraeum* (37.97%); this species was found abundantly in the Sangihe-Talaud waters. Thoha and Fitriya (2010) found *Trichodesmium* sp. dominate 50-95% (4842 - 83,043 cell m^{-3}) in almost all research stations. The community structure of the surface was different from the thermocline layer. The thermocline layer was dominated by the genus *Chaetoceros*, especially *C. affinis*, as much as 16.84%. The second species abundant in the thermocline layer was *T. nitzchoides* 13.58%. Genus *Chaetoceros* has a live strategy to survive by forming cysts during resting stages (Trottet et al., 2018). *Thalassionema* sp. in the South China Sea tended to high abundance in the thermocline layer rather than the surface (Boonyapiwat 2000). The low thermocline layer in light caused differences in environmental conditions from the surface, causing some types of phytoplankton in thermoclines to have unique characteristics to live and develop.

Phytoplankton abundance that varies between stations and layers of water was influenced by temperature and salinity. In general, the temperature will be directly proportional to the abundance of phytoplankton. The optimum temperature supports phytoplankton metabolic activities for cell development. As the founder of this study, stations on the surface with the highest temperature have the highest abundance. Similar conditions also occur to thermocline layers that have an average warm temperature. The thermocline is a euphotic zone that has a limit of a depth of 150 m (Raymont et al., 1980). The thermocline layer can be penetrated by sunlight to support the growth of phytoplankton (Barnes and Mann 1991), but the intensity was limited. A deeper thermocline layer has lower light availability than a shallow thermocline layer. Therefore, phytoplankton at shallow thermocline depths can do photosynthesis better than deeper thermocline layers. This condition was evidenced by the higher abundance of phytoplankton in stations with a depth of 75-100 m compared to 120-150 m. However, two stations were affected by the highest salinity, namely stations 12 and 13, indicating that the abundance of phytoplankton was not directly proportional to salinity. This result was supported by Soedarsono et al. (2013), who found phytoplankton abundance at salinity 40 ‰ of 22.25 cell m^{-3} dropped dramatically to 2.8 cell m-3 at salinity 27.5 ‰. Salinity above the tolerance threshold of phytoplankton causes osmosis stress, which inhibits growth with ion loss, inhibiting the absorption of nutrients, and inhibiting cell movement. Phytoplankton that cannot tolerate high salinity will avoid this area for life. Phytoplankton can survive when only a few species are in extreme conditions by forming cysts or spores (Sachlan 1972). Inappropriate salinity will increase phytoplankton metabolic activity so that its survival will be high, which is supported by increased RNA synthesis and DNA replication (Skarlato et al., 2017).

The composition of phytoplankton species at each observation station affected the diversity of phytoplankton. The diversity values were an indicator of the stability level of the phytoplankton community against environmental disturbances. The phytoplankton diversity in the surface was classified into small categories (Krebs 1989) because most of the H' values were <1.00. The small m of diversity is an indicator occurrence of high ecological pressure. On the other hand, the value of H 'in the majority thermocline layer was> 1.00, which was included in the category of moderate diversity as an indicator of fairly balanced ecosystem conditions. The higher phytoplankton diversity of the thermocline layer was due to the phytoplankton community being dominated by species from Class Bacillariophyceae and Cyanophyceae, which favor low sunlight intensity (Sellers and Markland 1987). Chlorophyll synthesis in species from Class Bacillariophyceae and Cyanophyceae did not require intense light; even powerful light will damage Phyto-oxidative phytoplankton enzymes and cause phytoplankton to die (Wetzel 1975; Barnes and Mann 1991; Riyono 2007). High phytoplankton diversity values in the surface and thermocline layer tended to be found at stations close to the island (Station 13 - Talaud Island, Station 1 - Sangihe Island). Meanwhile, the lowest diversity values in the surface and thermocline layers were found at stations far from the island (Station 4). High phytoplankton diversity values at stations close to the island were caused by nutrient input from the mainland. Many species of phytoplankton need nutrients to increase growth. Meanwhile, stations far from the mainland have fewer nutrient inputs, so the diversity was low.

To conclude, the total abundance of phytoplankton on the surface was 10 times greater than the thermocline layer. The phytoplankton abundance ranges from 77,333 to 4,024,000 cell m-3 on the surface and 8,000 to 542,222 cell m^{-3} in the thermocline layer. The differences in water conditions affect the variations in the species of phytoplankton. The surface layer was dominated by *Leptocylindrus danicus, Trichodesmium erythareum*, and *Detonula converfacea;* meanwhile, the thermocline was dominated by *Chaetoceros affinis, Thalassionema nitzchioides*, and *Chertoceros dichaeta.* Environmental parameters of temperature, salinity, and depth influenced the abundance of phytoplankton. Temperature shows a stronger influence on phytoplankton in the surface layer.

Similar conditions were found in the shallower thermocline depths (75-100 m), and areas with relatively high temperatures (21°C) have relatively high abundance. But the abundance of phytoplankton in the thermocline layer will be inversely proportional to salinity.

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Inventory of mangroves in Katunggan Coastal Eco-Park, Sultan Kudarat Province, the Philippines

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Abstract. *Mangaoang CC, Flores AB. 2019. Inventory of mangroves in Katunggan Coastal Eco-Park, Sultan Kudarat Province, the Philippines. Bonorowo Wetlands 9: 65-70.* The coastal wetlands of the Philippines are dominated by mangrove ecosystems and are experiencing different forms of threats, particularly anthropogenic activities. The local government unit of Lebak in the Province of Sultan Kudarat and non-government organizations envision rehabilitating and conserving mangrove forests, but no research has been done. Thus, this study was conducted to document different mangrove species, which will serve as a baseline in developing conservation and rehabilitation strategies. Purposive sampling was done, and morphological characteristics of each species were examined for identification. A total of 29 mangrove species belonging to 14 families were identified. These are threatened species, including *Ceriops zippeliana, Avicennia rumphiana,* and *Camptostemon philippinensis*. It was also noted that the local community is aware of the importance of mangroves. Still, not all have concerns about the government's conservation and protection policies. Despite the decreasing status of Philippine mangroves, local exploitation and anthropogenic pressures, such as aquaculture, are still uncontrolled. Therefore, the presence of threatened species implies that the area needs to be prioritized in conservation and rehabilitation activities with the joint effort of both the government and local communities to save and protect this mangrove ecosystem.

Keywords: Baseline, conservation, identification, mangroves, restoration

INTRODUCTION

Governments are promoting rehabilitation and restoration of mangrove ecosystems, non-government organizations, and aid agencies across different parts of Southeast Asia (Thorhaug 1990; Sukardjo and Yamada 1992; Saenger and Siddiqi 1993; Kaly and Jones 1998) with efforts that have met succession with necessary estimates of planted tree survival (e.g., Lewis 1990; Saenger and Siddiqi 1993; Calumpong 1994; Pomeroy et al. 1996; Primavera and Agbayani 1996; Erftemeijer and Lewis 1999). The degree to which mangrove planting actually facilitates the restoration of diverse and structurally complex forests similar to their natural precursors is little examined. However, considerable literature on this "catalytic" effect in upland forest plantations (Parrotta and Turnbull 1997).

The Philippines, as an archipelago, has over 7,100 islands, which is bordered by 36,300 km of coastline along seagrass beds, coral reefs, and even mangrove forests (Benecario et al. 2016). The biodiversity of mangroves and mangroves as an ecosystem has been increasingly attracting greater interest because of the highly productive, extremely sensitive, and fragile environment (Donoso 2018). Mangroves comprised of only 65-69 species are well known for their floral diversity of vascular plants that have several specific adaptations to the vibrant coastal setting (Kathiresan and Bingham 2001). Spalding et al. (1997) and Spiers (1999) stated that less than half of the remaining mangrove ecosystems are now existing to date and continue to be in degraded conditions (UNEP 2004; MAP 2005). The continued decline of the mangrove forests is due to numerous anthropogenic disturbances like conversion to agriculture, aquaculture, tourism, urban development, overexploitation (Alongi 2002; Giri et al. 2008), and even natural disasters. According to studies, 35% of mangroves had been lost from 1980 to 2000 (MA 2005), and the mangrove forests have been declining at a faster rate than either coral reefs or tropical forests (Duke et al. 2007). The rapid rise of sea level could also be the greatest threat to mangroves (Gilman et al. 2008). As a result, important ecosystem goods and services of mangrove ecosystems such as natural barrier of ocean waves, carbon sequestration, and the biodiversity contained in mangrove forests will be diminished or lost (Duke et al. 2007) even in the Philippines.

According to the latest Philippine mangroves checklist by Primavera (2006), there are at least 32 species known and found in the Philippine coastal areas. Consequently, a mangrove reforestation effort exists in the Province of Sultan Kudarat in Mindanao, Philippines. This is specifically located in the coastal Barangay Taguisa, municipality of Lebak, Sultan Kudarat. Barangay Taguisa, in cooperation with the Department of Environment and Natural Resources (DENR), has developed mangrove restoration and conservation programs to protect the area. This mangrove forest covers at least 1000 hectares of mangroves, but no record of mangrove species had been

published. Thus, this study was conducted. The data generated in this study will give a complete list of the species thriving in the area. It will also provide reliable baseline information for determining threatened species, which could be used to strengthen further the efforts being done in the conservation and protection of this ecosystem.

MATERIALS AND METHODS

Study period and location

The study was conducted during the summer between April-May 2018 in Katunggan Coastal Eco Park in Sultan Kudarat. one of the provinces in the Philippines situated in mainland Mindanao. During these months, most of the mangrove species in the area are in flower, which helped the researchers identify. Lebak, a municipality in the western Sultan Kudarat which faces the Celebes Sea, harbors both mountainous and coastal areas. Barangay Taguisa (A barangay is referred to as the smallest administrative division in the Philippines and is native to the Filipino term village, district or ward), a coastal community of Lebak, is where an eco-park can be found, which is part of the 1,000-ha mangrove forest (Figure 1). The Local Government Unit manages the Katunggan Coastal Ecopark in partnership with the Department of Environment and Natural Resources (DENR).

Sampling and inventory of mangrove species

The inventory of mangrove species was done through purposive sampling and walking on the park and in other parts of the mangrove forest in Barangay Taguisa, Lebak, and staff from the Municipal Environment and Natural Resources Office (MENRO) of the said municipality. Field notes were taken, and preliminary identification of mangrove species was made in the area. Morphological characteristics of leaves, flowers, and propagules were noted and used to identify species. Key guides such as the Field guide to Philippine Mangroves by Primavera 2006 and other published work were used. Photographic documentation was also employed to identify the species further. Voucher specimens were also collected.

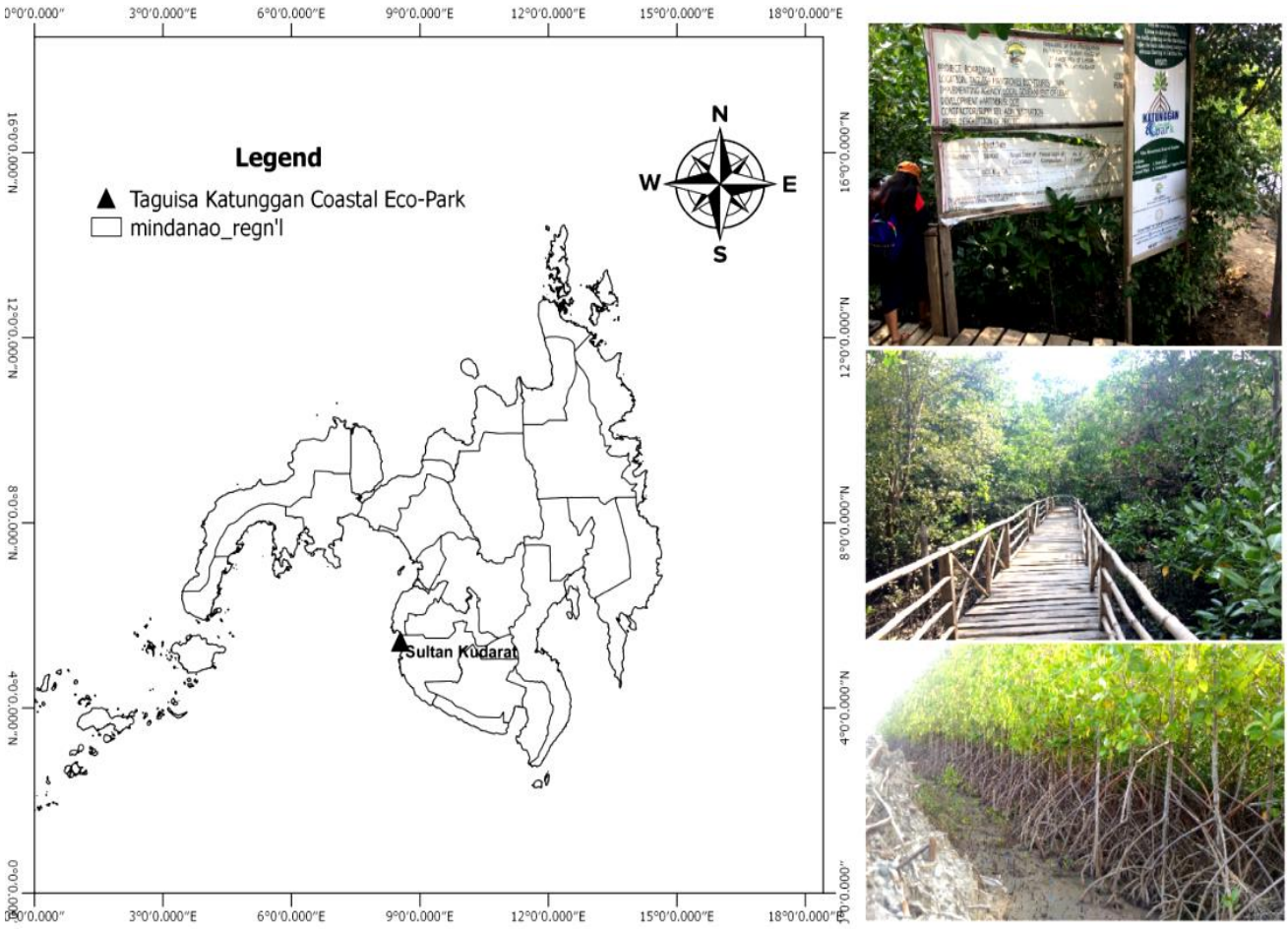

Figure 1. Map of the study site in Taguisa, Lebak Sultan Kudarat, Mindanao, Philippines

Table 1. List of mangrove species recorded during the survey in Taguisa, Coastal Eco-Park, Lebak, Sultan Kudarat, Philippines

Family	Species	Common name	Distribution	Ecological status
Fern				
Pteridaceae	*Acrosthicum aureum* L	Lagiwliw, ragoyhoy	Found in tropical and sub-tropical areas around the world	LC
	Acrostichum speciosum Willd.	Lagolo, palaypay	American Samoa; Australia; and Southeast Asia	LC
Vascular plants				
Acanthaceae	*Acanthus ebracteatus* Vahl	Lagiwliw, ragoyhoy	Widespread across Southeast Asia, including the Philippines	LC
	Acanthus volubilis Wall.	Lagiwliw, ragoyhoy	Widespread across Southeast Asia, including the Philippines and China, and Taiwan	LC
Arecaceae	*Nypa fruticans* Wurmb	Nipa, sasa	Throughout the Philippines	LC
Avicenniaceae	*Avicennia alba* Blume	Bungalon, apiapi, miapi	Southeast Asia, including the Philippines, Australia, and the Pacific islands	LC
	Avicennia marina (Forsk.) Vierh	Bungalon, apiapi, miapi	Southeast Asia, including the Philippines, Australia, New Zealand, East Africa, India, Pacific Islands	LC
	Avicennia officinalis L	Bungalon, apiapi, miapi	Southeast Asia, including the Philippines, New Guinea, and southern Australia	LC
	Avicennia rumphiana Hallier	Bungalon, apiapi, miapi	Southeast Asia, including the Philippines and Papua New Guinea	Vul
Bignoniaceae	*Dolichodrone spathacea* (L. fil.) K. Schum	Mangrove trumpet tree	Southeast Asia, including the Philippines. It is also found in the northeast tip of Australia and Papua New Guinea, and Palau.	LC
Bombacaceae	*Camptostemon philippinensis* (S.Vidal) Becc.	Gapas-gapas	This species is patchily distributed in Indonesia (Borneo and Sulawesi) and the Philippines.	En
Combretaceae	*Lumnitzera littorea* (Jack) Voigt	Tabao	Throughout the Philippines. Also tropical Asia, to Australia and Polynesia	LC
	Lumnitzera racemosa Willd.	Culasi	Luzon (Rizal, Bataan, Quezon) Mindoro, Panay, Negros, Cebu and Mindanao. Also eastern tropical Africa, Australia and Polynesia	LC
Euphorbiaceae	*Excoecaria agallocha* Linnaeus	Buta-buta	Throughout the Philippines. Also India, Malaysia, Australia, and Polynesia.	LC
Lythraceae	*Pemphis acidula* J.R. & G. Forst.	Bantigi	Throughout the Philippines. Also, tropical Africa tropical Asia.	LC
Meliaceae	*Xylocarpus granatum* Koen.	Tabigi	Throughout the Philippines. Also, East Africa, India, and some countries in Southeast Asia.	LC
	Xylocarpus moluccensis (Lamk.) M. Roem.	Piag-ao	Luzon (Quezon and Bataan), Palawan, Mindoro, Mindanao and the Sulu Archipelago. Also, Madagascar to India through Malaysia to Polynesia	LC
Myrsinaceae	*Aegiceras corniculatum* (Linnaeus) Blco.	Saging-saging, tinduk-tindukan	Batanes islands to Palawan, Mindanao and Sulu Archipelago. Also, Sri Lanka, India to southeastern China throughout Malaysia to Australia.	LC
Rhizophoraceae	*Bruguiera cylindrica* (Linnaeus) Blume	Pototan-lalake	Throughout the Philippines. Also, India, Southeast Asia through Malaysia and Australia	LC
	Bruguiera gymnorrhiza (L) Lamk.	Pototan-busain	Throughout the Philippines. Also Africa, Madagascar, Seychelles, Southeast Asia, through Malaysia, Australia, and Polynesia	LC

	Bruguiera parviflora (Roxb.) W. & A. ex Griff	Langarai	Throughout the Philippines. India, Southeast Asia, Australia, and New Caledonia.	LC
	Bruguiera sexangula (Lour.) Poir.	Pototan	Widespread and found in Asia, Northeast Australia, Papua New Guinea, and the Solomon Islands. This species is restricted to Hainan Island in China, where it is uncommon.	LC
	Ceriops zippeliana (Griff.) Ding Hou	Baras-baras	Throughout the Philippines. Also India, Southeast Asia, through Malaysia and Australia.	NT
	Ceriops tagal (Perr.) C.B. Rob.	Tungog, tangal	Throughout the Philippines. Also, tropical Africa, Madagascar, and most parts of Southeast Asia.	LC
	Rhizophora apiculata Blume	Bakhawlalake	Throughout the Philippines. Also, some parts of Southeast Asia, through Australia and the Pacific Islands.	LC
	Rhizophora mucronata Lamk.	Bakhaw babae	Throughout the Philippines. Extra-Philippine distribution including tropical Africa, Madagascar, and Seychelles	LC
Rubiaceae	*Scyphiphora hydrophyllacea* Gaertn.	Nilad, taguisa	Luzon to Palawan and Mindanao. Also in India, Southeast Asia, Australia, and New Caledonia.	LC
Sonneratiaceae	*Sonneratia alba* J. Smith	Pagatpat	Widespread in East Africa, Seychelles and Madagascar, India, Sri Lanka, and throughout Southeast Asia to tropical Australia, New Caledonia, Palau, the Federated States of Micronesia, Marshall Islands, Papua New Guinea, Solomon Islands, Vanuatu, Kiribati, and China (Hainan Island).	LC
	Sonneratia caseolaris (L.) Engl.	Pedada	Throughout the Philippines.	LC

Note: LC: Least Concern, NT: Near Threatened, En: Endangered, Vul: Vulnerable

RESULTS AND DISCUSSION

The survey and documentation of mangrove species in the Katunggan Coastal Eco-Park in Lebak, Sultan Kudarat identified a total of 29 species of mangroves belonging to 16 genera representing 14 families, including Acanthaceae, Pteridaceae, Myrsinaceae, Avicenniaceae, Bignoniaceae, Bombacaceae, Rhizophoraceae, Euphorbiaceae, Combretaceae, Arecaceae, Lythraceae, Sonneratiaceae, Rubiaceae and Meliaceae. Among the documented mangrove families, Rhizophoraceae had the most species with 8 under 3 genera while the least number of species was in Arecaceae, Bombacaceae, Bignoniaceae, Euphorbiaceae, Lythraceae, Myrsinaceae, and Rubiaceae with only one species per family. The complete list of species identified per family is presented in Table 1.

Most of the recorded mangroves in the area are widespread across Southeast Asia and neighboring tropical countries. The area also harbors some threatened mangrove species, which at least 3.45% of species are considered as Near Threatened (*Ceriops zippelliana),* 3.45 % species is vulnerable (*Avicennia rumphiana),* and an endangered species *Camptostemon philippinensis* is also 3.45% (Figure 2). The remaining 89.65% is considered Least Concern.

The mangrove forest in Katunggan coastal eco-park harbors 29 species of mangroves found in the Philippines, and this serves as the baseline data of the site. The high species present in the area can be incorporated with the local government's successful efforts in restoring and conserving this ecosystem. Numerous mangrove seedlings and saplings can also be observed in the area, which will be the next generation of the mangrove and be added to their population succession. Though the species number was lower compared to other mangrove ecosystems in the country, such as Pagbilao Bay in Quezon Province which has 37 species (Almazol et al. 2013); Panay with 34 species (Primavera et al. 2004), Guimaras with 30 species (Sadaba et al. 2009) and Davao Gulf with 30 species (Flores 2003), however, it is also considered higher compared to Bohol with 26 species (Mapalo 1992), Samar Island with 22 species (Mendoza and Alura 2001), Ibajay in Aklan Province with 22 species (Primavera 2000), Palawan with 22 species (Arquiza 1999), Danao Bay with 20 species (De Guzman 2004), Alabel and Maasim, Sarangani Province with only 12 species (Natividad et al. 2014), Bacolod, Lanao del Norte with only 11 species (Benecario et al. 2016) and in Hagonoy Davao del Sur with only 7 species (Jumawan et al. 2015).

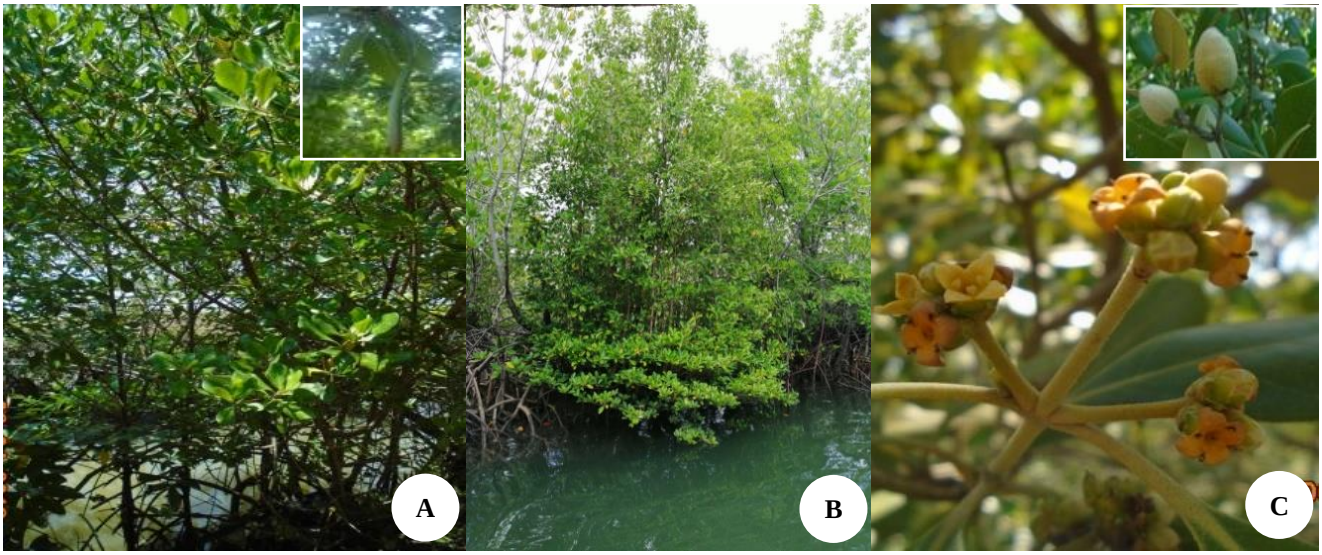

Figure 2. Photographs of mangrove species with conservation concern according to IUCN red list. A. *Ceriops zippeliana* (NT); B. *Camptostemon philippinensis* (EN), and C. *Avicennia rumphiana* (VU)

However, the number of species recorded in this study might be higher if a more intensive survey were conducted. The high species present in Katunggan Coastal Eco-park suggests that this area could be one of Mindanao's most important mangrove ecosystems and even in the country. The comparison between different mangrove areas only means that few mangrove sites have been explored and documented where most of the natural stands of mangrove can be found in the coastal areas of Mindanao. The study also noted that only one individual of *Pemphis acidula* was documented; this is probably because this mangrove species is locally threatened because of exploitation for decorative purposes such as bonsai. Locally known as bantigue bonsai, this mangrove species is listed in DENR Administrative Order 2017-11 as a threatened Philippine plant. It is also listed in the International Union for Conservation of Nature Red List of Threatened Species, where recent records of it being collected, sold, and transported are seized (Panay News, 2018). Even in the very alarming rate of anthropogenic disturbances, the fear of losing this threatened ecosystem is near to a possibility. And with the fact that it remains unexplored, we may yet lose another treasure trove without even knowing.

Based on observations and field notes of the study, the community is aware of the importance of the mangroves. Still, not all have concerns about the policies being implemented by the government to protect and conserve mangroves. There are still threats visible and can be observed in the area, such as rising numbers of agricultural fish ponds and degradation of mangroves or deforestation.

Conclusion and recommendation

The mangrove species present in Katunggan Coastal Eco-Park are indeed rich, and the data presented here qualifies the support of this claim. Successful local government restoration and conservation can be observed in the Katunggan coastal eco-park. These can be supported by the high species present and the observable number of saplings. Awareness of the ecosystem function of mangroves is limited only to the people who stay near the forest and those working in the eco-park. On the other hand, those who do not involve protection and conservation inflict destruction and disturbances. This became a big problem when implementing conservation efforts in the area.

The mangrove species richness in the area must serve as baseline information for further enhanced interventions and strong implementation of existing laws for its proper management and conservation. Local government officials and people in the community must work together to preserve this threatened ecosystem by performing each role that will subsequently lead to sustainable use of the resources, both flora, and fauna. The result of this study may be useful when developing a management plan to prioritize interventions for the conservation and appropriate utilization of services that the mangrove ecosystem could provide for them. This study recommends conducting ecological research on mangroves in the area, and an in-depth assessment of threats may be considered well.

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Ecology and control of *Typha* species in Hadejia-Nguru Wetlands, Nigeria

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Abstract. *Abdullahi YBY, Balarabe ML, Khan AU, Adamu AK. 2019. Ecology and control of* Typha *species in Hadejia-Nguru Wetlands, Nigeria. Bonorowo Wetlands 9: 71-91.* *Typha* proliferation causes several ecological problems, including transforming aquatic environments into terrestrial ones, interfering with various methods of catching fish, blockading river channels, and impeding navigation. The Hadejia Nguru wetland in Nigeria has an area of about 58,100 hectares, of which Typha species infested 35,000 hectares. The wetlands were divided into three sections, i.e., Upper, Middle, and Lower. In these segments, *Typha* species, water, soil, and sediment samples were collected, and the impact of *Typha* species on fish catch and distribution was assessed by splitting each segment into two sections: *Typha* uninfested and *Typha* infested. Each segment was separated into four sections for the biological control, each containing 2000 individuals of *Typha* interplant with 50kg, 25kg, 10kg, and no *Phragmites karka*, respectively. Each segment was manually separated into six sections as cutting *Typha* 15 cm, 10 cm, 5 cm below and 15 cm, 10 cm, 5 cm above the water. Each segment was divided into four sections for physical control, i.e., single black tarpaulin, double, triple, and no black tarpaulin. *Typha latifolia* and *Typha angustifolia* made up 64-70% and 30% of *Typha* species of the wetlands, respectivelyPhysico-chemical physics-chemical characteristics of water and sediment exposed substantial fluctuations, with PO4-P, NO3-N, and Mg concentrations ranging from 3.5-13.5 mg/L, 3-13 mg/L, and 1-10 mg/L, respectively. phosphate-phosphorus concentrations in the sediment ranged from 6.5 to 16 mg/kg, nitrate-nitrogen concentrations from 6 to 14 mg/kg, and organic matter concentrations from 4 to 12 mg/kg. The results also showed that high PO4-P, NO3-N, and Mg concentrations in water promote *Typha* development and proliferation. Sediment phosphate-phosphorus, nitrate-nitrogen, and organic matter concentrations showed a similar trend. The concentrations of water PO_4-P, NO3-N, Mg, and sediment PO_4-P, NO3-N, and Organic matter throughout the three segments revealed that the upper channel had a higher concentration than the middle, and the middle course had a higher concentration than the lower course. The distribution of *T. latifolia* and *T. angustifolia* followed the same pattern. The influence on fish catches and distribution revealed that open water had the maximum quantity and weight of fish taken, ranging from 83,167 to 173,026 kg and 14,402 to 59,355 kg, respectively, compared to *Typha* infested areas. Biological, manual, and physical strategies were used to control the spread of *Typha*. Biological control with *P. karka* reduced *Typha* species proliferation by 25%, with manual cutting at 15 cm below water level accounting for 95% of overall control. *Typha* proliferation was reduced by 54% when a black tarpaulin provided shade. Cutting at 15cm below the water's surface provided the finest control. Farmers should be educated on proper farming practices, particularly those that include manure rather than inorganic fertilizer. The optimum time to control *Typha* is when their density is low during the wet season.

Keywords: Control, ecology, Hadejia-Nguru Wetlands, *Typha*

INTRODUCTION

According to Mitsch and Gosselink (1986), Wetlands are places that transition between terrestrial and aquatic ecosystems and have a water table at or near the surface or are covered in shallow water (Prasad et al. 2002). Wetlands are gaining in popularity due to their numerous benefits to the environment. This includes a wide range of direct and indirect uses (Acharya 1998), such as (i) water retention during dry periods, keeping the water Table high and relatively stable; (ii) flood mitigation, and (iii) trapping of suspended solids and attached nutrients, resulting in streams flowing into lakes via wetland areas transporting fewer suspended solids and nutrients to the lakes than streams flowing directly into the lakes (Prasad et al. 2002). Wetland water quality is often impacted when wetland systems are removed due to urbanization or other circumstances (Barbier 2002).

Wetlands are also significant for wildlife because they provide resting and stopping locations for migrating and resident species, as well as refuges (Lameed 2011). Like any other natural environment, Wetlands play a vital role in maintaining species diversity. Different values include the use of wetlands for home and agro-industrial water supply (Ibrahim and Chiroma 1998), harvesting wetland bio-resources including fish and plants, and the role of wetlands in groundwater recharge and discharge (Ibrahim and Chiroma 1998). (Yahaya et al. 2010). The Hadejia-Nguru Wetlands, which cover 58,100 hectares and are designated as a Ramsar Site, are of international importance on the Ramsar wetlands. Wetlands are vital for water birds as a breeding area and a water source. The projected waterbird population ranges from 200,000 to 325,000, with about 377 bird species seen in the wetlands, including the near-threatened pallid harrier and great snipe species (Sanusi and Daura 2007). Drought struck the northeast of Nigeria in the early 1960s and 1970s, and to alleviate the situation, a water project was expanded

upstream. As a result, the Tiga Dam was built, and aquatic weeds, particularly *Typha* species, became a nuisance, harming the social and economic well-being of the people who rely on the wetlands. Blockage of river channels is one of the issues connected with *Typha* weeds, but it also provides breeding and nesting habitat for Quelea birds (Sanusi and Daura 2007). The Hadejia Nguru Wetlands (HNWs) are located in north-eastern Nigeria's Sahel zone. The area is a floodplain marsh with permanent water bodies and sections flooded seasonally. Approximately 40% of the wetlands remain wet throughout the year, resulting in *Typha* mats (currently over 200 hectares, compared to 550 ha in 1999). (Sanusi and Daura 2007).

The frequency of *Typha* species was first discovered in the Lake Chad Basin. It was classified as one of the basin's seven top regional environmental issues by Transboundary Diagnostic Analysis (TDA) (Sanusi and Daura 2007). Variability in the hydrological regime and fresh water availability, water pollution, decreased biological resource viability, ecosystem loss, alteration, and sedimentation in rivers and water bodies are among the others. However, the Komadougou-Yobe subbasin (KYB), Chari Logone subsystem, and the lake itself are the most affected. *Typha* species and Quelea birds are two major invasive species in the KYB sub-system. Water hyacinth dominates the Chari-Logone system, while *Typha* species have taken over the lake. *Typha* is a water-loving plant that can multiply and become difficult to control in favorable conditions (i.e., shallow, persistently flooded regions), making it invasive. It out-competes practically all other plants in such conditions.

The invasion of *Typha* species has posed one of the most severe challenges to the economy and ecology of the Hadejia-Nguru Wetlands and other portions of the Hadejia-Jamaare-Komadugu-Yobe Basin (HJKYB) in general in recent years. *Typha* has recently taken over river channels, lakes, and fadamas in the wetlands and several hectares of farmland and prospective grazing pastures. Over 35,000 hectares of potential farming and grazing fields have been taken over by the *Typha* species in the Marma Channel and Nguru Lake (a portion of Hadejia-Nguru Wetlands), for example, where *Typha* invasion is especially severe. On the other hand, it has contributed to the desiccation of the Burum Gana Channel, which has hampered around 60% of dry season irrigation crops. Furthermore, *Typha* serves as a haven for vast flocks of Quelea birds (another invasive bird species in the basin), which are a nuisance to cereal crops (Sanusi and Daura 2007).

The most significant environmental impact of weed infestation is channel blockade and channel diversion in some instances. This has resulted in cases of simultaneous channel desiccation and inundation in HNWs, with the net result of lost livelihoods, poverty, and resource use disputes (Lameed 2011).

The goal of the study was to look into the diversity and influence of *Typha* species in the Hadejia-Nguru Wetlands, as well as to provide management advice for long-term ecology, with the following specific objectives: (i) To identify the *Typha* species that live in the Hadejia-Nguru Wetlands and their relative abundance monthly over two years. (ii) To determine the monthly physicochemical characteristics linked with *Typha* species proliferation for two years. (iii) To assess the soil, nutritional condition, and qualities that support *Typha* species growth every month for two years. (iv) To assess the impact of *Typha* species on fish catches and distribution during two years. (v) To determine the most effective management strategy for the Hadejia Nguru wetlands community.

MATERIALS AND METHODS

The study site and background to the research location

The Hadejia-Nguru Wetlands (HNW) is located in north-eastern Nigeria and covers around 58,400 ha. They are located between the latitudes of 12º40'N and 13º60'N and the longitudes of 10º20'E and 11º00'E. (Figure 1). Hadejia-Nguru Wetlands (HNW) is bordered by a flood plain consisting of a network of channels and pools that produce a whole pattern of constantly and seasonally flooded land and dry land (Hollis et al., 2003).

Field methods

Sampling stations

The wetlands were separated into three divisions based on preliminary study findings: a topography of the wetland system, human settlement, and fishing activity (Figure 1). The sampling stations were:

Upper course. Between N12 49'16.1' and E l0 24' 21.5', Punjamu is near the entrance to the marsh where water drains from the Marma canal. It is located at 343 meters above sea level and has a high concentration of aquatic macrophytes, particularly *Typha* species, due to higher water, soil, and sediment nutrients.

Middle course. Badun is situated near the Dabar Magini town, between N12 50' 27.9' and El0 24' 08.1', at an altitude of 334m. There is a lot of fishing and farming going on there. In addition, the residents of this community work in the potash exploration and refinement industry.

Lower course. This location, between coordinates N12 49' 40.7' and El0 24' 21.1' with a height of 341m above sea level, has minimal human activity and few *Typha* species.

Identification of *Typha* species in Hadejia-Nguru Wetlands

Plants were sampled in the study region, and *Typha* species were identified using the Aquatic Plant Information System (APIS 1996) (Table 1).

Percentage occurrence of *Typha* species

Line transects were used to establish the percentage occurrence of each *Typha* species. The Typha species were counted every five meters from the shoreline to the open water. According to Titus, the percentages of occurrence of each species of *Typha* were calculated using the formula below (2003). The population density is defined as the number of people per unit area divided by the total land area. The proportionate representation of a species in a given ecosystem is called abundance.

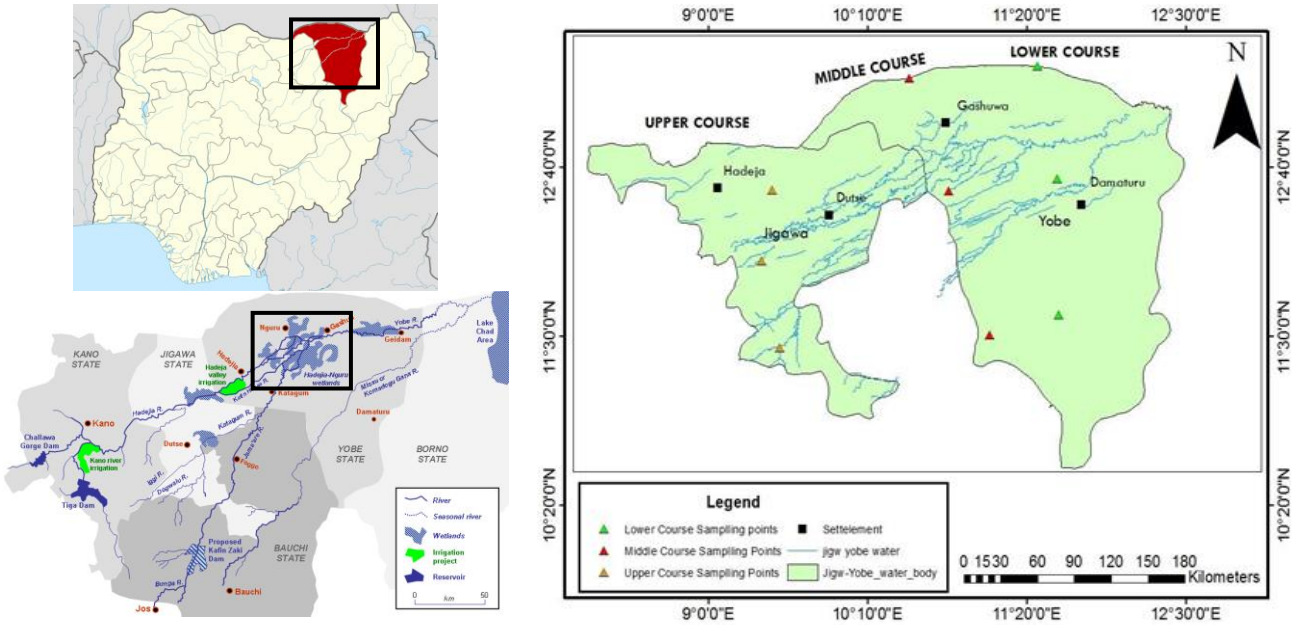

Figure 1. Map of Yobe State, Nigeria showing Hadejia-Nguru Wetlands (*left*) and area of the sampling points and site locations (*right*)

Table 1. Morphological features for identifying *Typha* species (APIS 1996)

Parameter	*T. latifolia*	*T. angustifolia*	*T. xgluca*	*T.dominigensis*
Appearance	Coarse stout	Slender	Either	Slender
Leaves x-section	Flat 8-15	Convex on back 5	Convex on back 6-12	Convex on back 6-12
Width of leaves in mm sheaths	Tapering	Auriculate	Auriculate	Tapering
Length between female and male	Non	5-12cm	0-4cm	0.7-4.5cm
Pith color at the base	White	White	Yellow buff	White
Female flower bract	None	Darkbrown blunt	Non rarely like ang and dom	Light brown ovate and apiculate

$$\text{Percentage of species (\%)} = \frac{\text{The area covered by each species}}{\text{Whole Total area of the habitat}} \; x\; 100$$

Determination of *Typha* species density

The following approach was used to determine the density of *Typha* species, as stated by Smith (2009): Each sampling location was divided into 50mx50m areas infested with *Typha* species. For two years, the number of each species in a 2500m^2 area was recorded and correlated with each physicochemical parameter of water, sediment, and soil nutrients.

Collection of water samples

A Van Dorn Water Sampler was used to take water samples in the wetlands, and it was dropped into the water with a graduated rope at a higher water level. Temperature, transparency, electrical conductivity, and pH were measured at the sample stations. Before being transported to the NIFFR hydrobiology laboratory for analysis, the water was transferred into clean sample bottles.

Determination of physical characteristics of water

Determination of temperature

A mercury thermometer calibrated to the nearest 1oC was used to determine the air and water temperature. Holding the thermometer above the water for 5 minutes until it stabilized was used to take air temperatures. Before recording the temperature, the thermometer was lowered into the water inclined at 10cm for 5 minutes to allow for equilibrium.

Determination of Transparency

The APHA-recommended Secchi disc was used to measure transparency in the field (2005). To estimate the openness, a Secchi-disc with a diameter of 25cm was lowered into the water with a measuring tape until it vanished from view, and the measurement was recorded as P1. The Secchi-disc was then removed, and the depth of reappearance was measured and recorded as P2, with the transparency computed using the formula below: Transparency = P_1+P_2/2.

Electrical conductivity (EC)

The electrical conductivity of soil samples was measured using a HANNA digital Electrical Conductivity (EC) meter with a soil-water ratio of 1-2 (w/v) (HI98129 model). 0.1M KCI was used to calibrate the electrical conductivity meter. In 100ml plastic containers, ten grams (10g) of 2mm sieved air-dried soil samples were weighed, and 20ml of distilled de-ionized water was added. After

that, the mixes were agitated for 30 minutes. The soil suspensions were then left undisturbed for 30 minutes. After that, the EC meter's electrode was put into the settled suspension. The EC of soil samples was determined. Micro-ohms/cm (s/cm) was used to test electrical conductivity.

Determination of chemical characteristics of water

Determination of pH

Each sampling station's surface water was collected in plastic bottles, and the pH was calculated at 25°C using a 3015 model's pH meter. The electrode was dipped into the water sample, and a reading was taken and recorded. The pH meter was standardized with a buffer solution at pH 4.0, 7.0, and 9.0.

Dissolved Oxygen (DO)

The modified Winkler's method was used to determine the amount of dissolved oxygen (APHA, 2005) 250ml of water was collected from the designated sampling sites. Amber sampling bottles and filled up to the full. On the spot, 2 mL of standard Manganous sulfate solution and 2 mL of alkaline-iodide solution were added, and the contents were agitated for 10 seconds. To avoid air being trapped in the sample, 2ml concentrated Sulphuric acid (H2SO4) was added, and the stopper was replaced immediately. The bottle was shaken for a few seconds more to dissolve the precipitation. The samples were subsequently transported in an icebox to the NIFFR hydrobiology laboratory.

With sodium thiosulphate 0.2000N, the produced solution was titrated until the sample changed color from yellow to colorless. The number of digits from the counter window was read, and the value was multiplied by 0.1 to get the dissolved concentration in mg/L (APHA, 2005). A D.O. meter, model HI9142, was also used to measure DO.

Biological oxygen demand (BOD)

The water sample was incubated in 250ml BOD bottles at 20ºC for five days after the initial dissolved oxygen (DO) was calculated using the modified Winkler's method described above. On the fifth day, DO was determined on the incubated water sample once more, and BOD_5 (mg/L or mgL-1) was calculated as DO_1-DO_5 (mg/L). Where DO_1= Dissolved oxygen concentration before incubation. DO_5= Dissolved oxygen concentration after 5 days incubation (APHA, 2005) crosschecking using DO meter HI9142 for comparison.

Water phosphorus

Total phosphorus in water was measured using the APHA method (2005). To transform all chemical properties of phosphorus into phosphates, the sulphuric acid-nitric acid digesting process was applied. 50 mL of water and 1 mL of concentrated solution were pipetted into a microkjedhl flask. H_2SO_4 and 5 mL concentrated solution HNO_3 were added to the mix. The combination was digested to a clear solution volume of 1 mL. The solution was chilled before adding 20 mL of distilled water. After neutralizing NaOH, the solution was prepared up to 100 mL with distilled water. 50 mL of the treated sample and 8.0 mL of the mixed reagent were pipetted into a volumetric flask. After complete mixing, the solution was allowed to sit for 10 to 30 minutes to produce the blue color. The sample's absorbance was measured at 880 nm on a 634 UV-visible spectrophotometer with a reagent blank as the reference solution. Extrapolation from a calibration curve utilizing standard phosphate-phosphorus concentrations was used to estimate total phosphorus content.

Water nitrate-nitrogen

Mackereth (1978) and APHA (2005) described the phenol sulphonic acid technique, which was used in this study, as Fifty (50ml) of the sample was measured into a Kjeldahl flask, 0.3g of magnesium oxide was added, 0.4g was also added, the flask was attached to the distillation machine, and the sample distills were collected. The distillate had a greenish hue to it. The distillate was then titrated with standard acid, and a green to pink color was seen; the titer value was recorded from the burette for nitrate-nitrogen calculation, as shown below:

$$N = \frac{Titre\ value\ x\ 100}{Mills\ of\ aliquot\ (50ml)}$$

The result was recorded as the concentration of nitrate in mg/L

Water magnesium and calcium

Twenty's (20 mL), 5 mL buffer solution, 3 drops, potassium cyanide, potassium ferrocyanide, hydroxylamine, hydrochloride, triethanolamine, and eriocromy black-T indicative were added for each of the following reagents. The solution turned purple, and EDTA 0.01M was added to titrate it.

The endpoint turned a permanent blue color, and the titer value was recorded, along with the Magnesium ion concentration, which was calculated using the formula:

$$Mg + Ca = \frac{Titre\ value\ x\ 0.01}{Mills\ of\ aliquot} x1000$$

The results were equivalent to Ca + Mg. As a result, the amount of calcium was subtracted from this value to get the Magnesium value.

Sediment determination

Sediment pH

In a beaker, 10 g of dried sediment were weighed and mixed with 10 mL of distilled water. After 30 minutes of stirring with a glass rod, the liquid was left undisturbed for another 30 minutes. The pH level was measured and recorded (Udo and Ogunwale 1986).

Electric Conductivity (EC)

The EC was calculated by immersing the electrode of the conductivity meter in the sample and recording the EC in s/cm. Ten (10g) of dried silt was weighed and put into a

beaker with 50 mL of distilled water (Udo and Ogunwale 1986).

Sediment nitrate-nitrogen

Two (2g) desiccated sediment was weighed and placed in a Kjeldahl flask with 20 mL concentrated Sulphuric acid. Digestion was carried out in the fume cupboard's digestion chamber for one hour.

After allowing the contents to cool, distilled water was added to dilute the digested sample to a volume of 50 mL. Distillation was performed by placing 10 mL of the digested material in a macro Kjeldahl flask and adding 30 mL of distilled water. Then 20 mL of 40% NaOH was added, and the flask was coupled to the distillation machine. Under the condenser, a boric acid indicator was inserted. Then, against 0.01 molar H_2S0_4, titrated. The mixture turned from green to pink, and the titer value was recorded so that the amount of nitrogen in the sample could be calculated using the formula:

N = titre value x 0.01 molar x 0.014 x 50 x 1000/weight of sediment x mills of aliquot. The concentration was calculated as nitrate-nitrogen in g/kg.

Sediment Calcium Ion Exchange Capacity (CEC)

Five (5) grams of silt were weighed and transferred to a conical flask containing 20 mL of ammonium acetate solution, saturated overnight. The filtrate was collected in a conical flask after the mixture was filtered with a funnel and filter paper. A 30 mL solution of ammonium acetate was also added to wash the sediment residue from the flask, which was then filtered with paper and funnel. Na, K, Ca, and Mg was measured in the filtrate solution. A flame photometer was used to determine Na and K. The Atomic Absorption Spectrophotometer (AAA) determined Ca and Mg.

Sediment phosphate-phosphorus

Two (2) grams of silt were weighed and put into a conical flask, along with 7 mL of phosphorus extraction solution, and mechanically agitated for 30 minutes. The filtrate was collected after the mixture was filtered. As a result, 2 mL of the filtrate was added to a 50 mL volumetric flask, followed by 2 mL of ammonium molybdate, 30-40 mL of distilled water, 1 mL of fresh diluted stannous chloride, and distilled water. The spectrophotometer was used to measure the color intensity at 660 wavelengths; the reading was obtained and used to determine the phosphorus concentration as follows:

$$P = \frac{Reading\ x\ conversion\ factor\ (0.61) x\ dilution\ factor\ (25)}{Atomic\ weight\ of\ phosphorus}$$

The value was recorded as phosphorus in g/kg

Sediment total organic matter content (TOC)

Researchers employed Agbeti and Smol's (1995) approach to calculate TOC. In a 250 mL conical flask, 1g of finely ground silt sample was added. After that, 10 mL 17N K_2 Cr_2 O_7 and concentrated H_2SO_4 were added, and the mixture was allowed to cool for 30 minutes. Whatman filter paper N01 was used to filter the suspension. 1-5 drops of ferroin indicator were added, then titrated with 0.4N $(NH_4)_2$ $SO_4.6H_20$ until the endpoint changed from dark green to red. The following formula was used to determine the TOC:

$$Total\ organic\ matter\ content\ (TOC)\ (g) \frac{meg\ of\ Cr_2O_2\ meqfe - NH_4\ SO_4 x4}{Wt.of\ sample}$$

Sediment particles size distribution and textural classification

The hydrometer method was used to determine the particle size (Bouyoucos 1951). A 250 mL plastic beaker was filled with fifty grams (50g) of a 2mm sieved soil sample. Fifty milliliters (50 mL) of 6% H2O2 was added, covered with glass, and set on a water bath to oxidize the organic materials (indicated by the presence of effervescence). The beaker was taken out and set aside to cool. The contents were placed in a dispersion cup with 400 mL of distilled water. A 100 mL Calgon solution was added, and the mixture was churned for 10 minutes with a glass rod. The suspension was poured into a setting cylinder, filled to the 1-liter mark. The mouth of the cylinder was then sealed with a rubber stopper, and the cylinder was vigorously shaken for 1 minute. The cylinder was then placed on a table, and after four minutes, the first reading was taken. The suspension's temperature was also measured and recorded. The suspension was left alone for a while. Two hours after the first shaking ended, the hydrometer was reinserted into the suspension, and the reading was taken for the second time.

The sand, silt, and clay compositions were calculated, and the texture of the soil sample using the textural triangle. The following formula was used to calculate the sand, silt, and clay composition (percentage):

% Silt= d-g/ax100
% Clay= g/ax100
% Sand= 100-(%Silt+%Clay)

Where;
d: corrected hydrometer reading at 4 mins
g: corrected hydrometer reading at 2 hours observation
a: weight of the soil sample (g)

Verma and Agarwal (2007) reported that the soil texture triangle was used to classify the soil samples based on the samples' relative percentage composition of clay, silt, and sand.

Soil chemical determination

Soil phosphate-phosphorus

Five (5) grams of dry soil was weighed and placed into a tube with a stopper, and 35cm^3 of extraction solution was added (with a pipette). After 1 minute of shaking, the suspension was filtered into a dry bottle. Back through the filter with the solution.

Ten (10) cm^3 of the filtrate was transferred to a dry 100cm^3 beaker with a 25cm3 ammonium molybdate reagent. A 5cm^3 stannous chloride dilutes solution was

added and stirred. The absorption was measured at 890 nm on a spectronic 20 after 10 minutes of color development.

Soil nitrate-nitrogen

The chemical composition of the soil was determined according to Gee and Bauder's guidelines (1986). In a 500 mL Kjeldahl flask, one (1) gram of 100 mesh dirt was weighed. For around 30 minutes, four drops of water were left to stand. 5g Kjeldahl catalyst combination and concentrated sulphuric acid were added. Digestion was carried out in the digestion chamber under the fume cupboard for one hour.

After cooling the contents, distilled water was added to dilute the digested sample to 50 mL. Distillation was carried out by adding 30 mL of distilled water to 10 mL of digested sample in a macro Kjeldahl flask. After that, 20 mL of 40% NaOH was added, and the associated flask was placed on the distillation machine. Under the condenser, a boric acid indicator was inserted. The sample was then heated to distillate, which was then titrated against 0.01 molar H2S04, the mixture-colored pink, and the titer value was recorded to calculate the quantity of nitrogen in the sample using the formula below:

N = titre value x 0.01 molar x 0.014 x 50 x 1000/Weight of sediment x mills of aliquot.

The concentration was calculated as nitrate-nitrogen in g/kg.

Soil organic carbon and organic matter determination

The method of Walkley and Black (1934), which IITA used, was used to determine organic carbon (OC) (1979). One (1) gram of dirt was weighed into 250 mL conical plasmin triplicates. Each flask received 10 mL of $0.02moldm^{-3}$ potassium dichromate, gently swirled to disseminate the soil, followed by 20 mL concentrated H_2SO_4. The flask was gently stirred to thoroughly and reagents completely. The mixture was then allowed to sit for 30 minutes on a glass plate before being diluted with 200 mL distilled water and 1 mL ferroin indicator, then mixed and titrated with $0.25moldm^{-3}$ ferrous ammonium sulfate. A blank titration was performed simultaneously but without the soil sample to determine organic carbon (OC) and organic matter (OM). The formula below was used:

% OC= (Blank titre-Actual title) X 0.3xf/weight of air-dried soil sample

Where: f = correction factor =1.33M = concentration ferrous ammonium sulphate% OM in soil=%) OC X 1.729

Soil calcium, magnesium

Five (5) gram of soil was weighed and put to a conical flask containing 20 mL of ammonium acetate solution, which was saturated overnight. The filtrate was collected in a conical flask after the mixture was filtered with a funnel and filter paper. A 30 mL ammonium acetate solution was also added to wash the sediment residue in the flask, filtered with paper and a funnel. Na, K, Ca, and Mg concentrations were determined using the filtrate solution. Impact of *Typha* species on fish catch and distribution in Hadejia-Nguru wetland

The Hadejia Nguru wetland was divided into three sampling sites, with each sampling site was split into A and B for collecting data. A is an uninfested area, whereas B is a *Typha-infested* area. For two years, experimental gillnet mesh sizes of 1; 1.5; 2; 2.5; 3; 3.5; 4; 5; 7; were set in the evening and checked early in the morning. Weighing and spring balances of various sizes measured the caught fish. Olaonsebikan and Raji (2004) identified fish species by hand. Dissolved oxygen (DO), biological oxygen demand (BOD), and the number of caught fish species were all recorded at the three sampling sites. Management Methods

Biological control of *Typha* species with varying weights of *Phragmites karka* (As noticed by Birnin-Yauri, 2009 in the field). *Typha* species infested plots were labeled A, B, C, and D. Two thousand (2000) *Typha* species per $2500m^2$ were randomly selected for the experiment in each sample station; (i) A: *P. karka* was interplanted with 50kg of *P. karka*. (ii) B: *P. karka* was interplanted with 25kg. (iii) C: 10kg of *P. karka* were interplanted with. (iv) D: Control studies used *Typha* species devoid of *P. karka*. For two years, the mortality rates of *Typha latifolia* and *Typha angustifolia* were recorded monthly.

Manual control

Manual control was carried out by the instructions of Nelson and Dietz (2006). A $2500m^2$ plot infected with *Typha* species was repeated three times, with two thousand stands of *Typha* species plants chosen at random in each sampling site A, B, C, and D. (i) A: shoots of *Typha* plants which were cut 5cm below the water's surface and immersed for two years. (ii) B. submerged shoots that have been cut 10cm below the surface of the water. (iii) C. shoots, cut 15cm below the water, and submerged. (iv) D: shoots which were cut 5cm, 10cm, and 15cm above the water as control experiments. For two years, the number of dead plants and regrowth were counted at three sites within the sampling site.

Physical control using shading

Linde et al. (1976) described how physical control was carried out. Three times a 50m by 50m plot infected with *Typha* species was duplicated (A, B, C, and D). For the experiment, each was counted and included two thousand *Typha* species. A 50mx50m plot infected with *Typha* species containing two thousand *Typha* species was covered with 100mx100m of black tarpaulin, marked A, B, C, and D in each sampling site. (i) A represents a single black tarpaulin; (ii) B represents a doubled black tarpaulin; (iii) C represents a tripled black tarpaulin; (iv) D is a control experiment with no black tarpaulin. The mortality rate of *Typha* species was measured in three locations inside the sampling station for two years.

Statistical analysis

Analysis of Variance (ANOVA) was performed on the data acquired using the Statistical Package for Social

Sciences (SPSS). Data were subjected to the Principal Components Analysis for physical and chemical parameters, soil nutrients, and sediment nutrients. The significance of the difference between *T. latifolia* and *T. angustifolia* was determined using analysis of variance (ANOVA). The fish's numbers and weights were captured in two distinct places: the Typha-free area (site A) and the *Typha*-infested area (site B). In terms of the influence of different *P. karka* weights, (i) A. *P. karka* (50kg) was tested. (ii) B. 25kg of *P. karka*, (iii) C. 10kg of *P. karka*, (iv) D. Free from *P. karka* as control. In terms of re-growth rate, there is a significant difference among (i) A. 5cm below the water level, (ii) B. 10cm below the water level, (iii) C. 15cm below the water level, and (iv) D. 5cm, 10cm, 15cm above the water level as control. To separate the means of the data collected, Duncan's Multiple Range Test was employed. The correlations between the physicochemical parameters of sediment, soil nutrients, and *Typha* species were determined using Pearson's product-moment correlation coefficient. The T-test was employed to see a significant difference between the two years assessed environmental and biotic variables. The significance level was chosen at ($P<0.05$).

RESULTS AND DISCUSSION

Typha species resident in Hadejia-Nguru wetland

Typha latifolia and *T. angustifolia* were the species of *Typha* identified in Hadejia-Nguru wetland over two years, with 65-70% and 30-35% occurrence, respectively, as shown in Figures 2 and 3. According to the findings, T. latifolia had a higher population than T. angustifolia. The inflorescence is an easy way to distinguish the two *Typha* species. In the inflorescence of *T. angustifolia*, the male and female reproductive components are separated. The male and female reproductive components in the inflorescence of *T. latifolia* are not separated. *T. latifolia* has narrow leaves, but *T. angustifolia* has broad leaves. The quantity of *T. latifolia* and *T. angustifolia* differed significantly (Table 2). In the interaction between *T. latifolia* and *T. angustifolia*, there was a substantial variation between Month and Sample, Month and Location, and Sample and Location. There is no significant difference between location and Season and Month x Sample ($P<0.05$).

Typha species density

The density of two *Typha* species was *T. latifolia*, followed by *T. angustifolia*. *T. latifolia* and *T. angustifolia* density ranged from 40 to 100, and 20 to 60 stands per $9m^2$, respectively (Figure 2).

Principal components analysis

Six variables were discovered to be responsible for the expansion of *T. latifolia* and *T. angustifolia* in the Hadejia-Nguru Wetlands, according to the results of principal component analysis. Water phosphate-phosphorus, Nitrate-Nitrate, Magnesium, Sediment phosphate-phosphorus, Nitrate-Nitrate, and organic matter were among them (Table 3).

Relationship between some physical and physicochemical parameters and *Typha* species density

Principal component analysis revealed that physical characteristics such as air temperature transparency and electrical conductivity (EC) have no relation to Typha species' growth in the Hadejia Nguru wetland. The air temperature ranges from 21 to 35 degrees Celsius, 0.4 to 1.9 meters, and 166 to 375 (s/l), respectively. Dissolved oxygen (DO) and biological oxygen demand (BOD) were measured and varied from 7.0 to 8.8, 0.9 to 8.84 mg/L, and 4.11 to 15.00 mg/L, respectively (Tables 4 and 5).

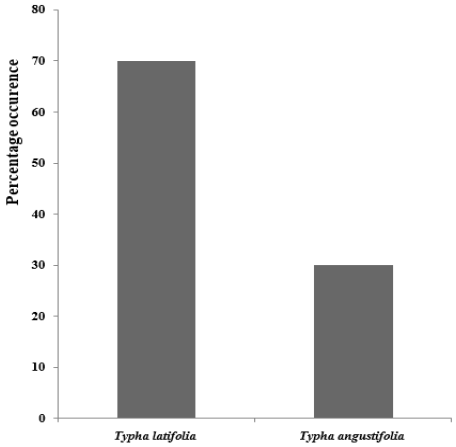

Figure 2. The percentage occurrence of *Typha latifolia* and *T. angustifolia* resident in Hadejia Nguru Wetland during dry season 2010 and 2011.

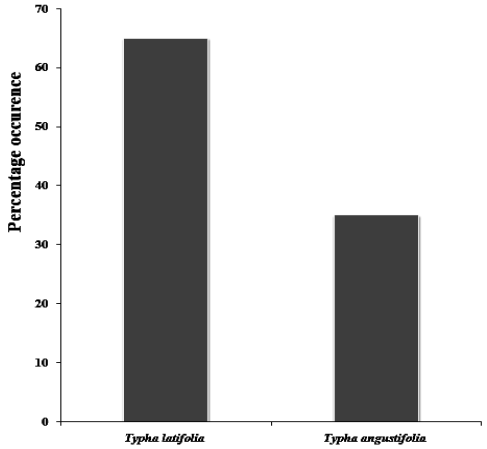

Figure 3. The percentage occurrence of *Typha latifolia* and *T. angustifolia* existing in Hadejia Nguru Wetland during wet season 2010 and 2011

Table 2. Analysis of variance between *Typha latifolia* and *T. angustifolia* density in the study area for two years (2010 and 2011)

Source of variation	DF	*Typha latifolia*	*Typha angustifolia*
TL (Treatment)	116	0.001*	0.003*
Month	11	0.004*	0.004*
Sample	2	0.002*	0.003*
Location	2	0.021*	0.025*
Season	1	0.008*	0.042*
Month sample	12	0.001*	0.372
Month location	22	0.0278*	0.001*
Sample location	4	0.0421*	0.042*
Location season	2	0.043*	0.367
Month sample location	44	0.021*	0.234
Sample location season	6	0.021*	0.041*
Error	206		

Note: * Significant; at (P<0.05).

Table 3. Principal component analysis of chemical characteristics of sediment and water from Hadejia-Nguru wetlands 2010 and 2011

Variables	Extraction	Main factors extracted
Phosphorus water	0.999065	Water Phosphorus
Nitrogen Water	0.999065	Water Nitrate
Magnesium	0.998774	Water Magnesium
Sediment phosphate-phosphorus	0.998233	Sediment phosphate-phosphorus
Sediment nitrate nitrogen	0.997911	Sediment nitrate nitrogen
Sediment organic	0.996365	Sediment organic matter

Table 4. The average physicochemical parameters extracted out by Principal Component Analysis in Hadejia Nguru wetland in 2010

Month water Temp. ºC	Air temp. (ºC)	pH	Transp. (m)	DO_2 mg/L	BOD mg/L	Conduct. (µs/l)
Jan. 10 16	21	7.8	1.8	9.24	10.05	328
Feb. 10 18	24	7.8	0.7	4.83	14.41	297
Mar. 10 17	24	7.7	0.9	3.15	9.53	285
Apr. 10 20	27	8.1	1.9	2.24	8.65	289
May 10 25	35	7.5	1.8	8.84	12.71	261
Jun. 10 21	33	7.6	1.7	1.55	11.46	302
Jul. 10 20	30	6.9	1.0	1.92	10.88	268
Aug. 10 15	21	7.0	0.4	0.91	7.53	260
Sept. 10 16	22	7.3	0.9	5.82	14.91	266
Oct. 10 22	26	7.8	1.4	2.64	8.72	268
Nov. 10 21	25	8.0	1.5	3.11	7.99	370
Dec. 10 18	24	7.4	1.7	3.15	4.11	375

Table 5. The average physicochemical parameters extracted out by Principal Component Analysis in Hadejia Nguru wetland in the year 2011

Month water temp. (ºC)	Air temp. (ºC)	pH	Transp. (M)	DO_2 mg/L	BOD mg/L	Conduct. (µs/l)
Jan. 11 15	21	7.8	1.8	9.24	8.05	308
Feb. 11 20	25	7.9	0.7	4.83	14.25	297
Mar. 11 19	22	7.7	0.9	3.15	9.52	185
Apr. 11 22	28	8.1	1.9	2.24	14.23	280
May. 11 25	35	7.5	1.8	8.82	15.00	266
Jun. 11 23	32	7.5	1.7	1.55	1.46	302
Jul. 11 20	30	6.5	1.0	1.92	9.89	268
Aug. 11 15	22	7.0	0.4	0.91	10.54	260
Sept. 11 14	22	7.3	0.9	5.82	9.92	166
Oct. 11 17	27	7.8	1.5	2.64	8.73	265
Nov. 11 21	25	8.0	1.6	3.11	9.89	276
Dec. 11 19	24	7.5	1.7	3.15	12.12	275

In terms of transparency, temperature, electrical conductivity, and pH, most aquatic plants can thrive in various conditions: biological oxygen demand (BOD) and dissolved oxygen (DO) (BOD). Similar trends were seen in soil texture and nutrient concentrations related to soil mineral leaching into the sediment. The elements that govern *Typha* species proliferation must be studied directly from the root and those that control photosynthetic activities.

Relationship between water phosphate-phosphorus and *Typha* species density

phosphate-phosphorus is necessary for organism growth and can be the nutrient that restricts water's primary production. Figure 4 shows the results of the phosphate-phosphorus concentration in the Hadejia-Nguru Wetlands. phosphate-phosphorus concentrations ranged from 3.5mg/L to 13.5mg/L. *T. latifolia* densities ranged from 60 to 100 stands per $9m^2$, and *T. angustifolia* densities ranged from 40 to 65 stands per $9m^2$. During the extreme dry season (April-May), high values of water phosphate-phosphorus were recorded, while lower values were observed during the rainy season (July-August). With phosphate-phosphorus concentration, the populations of *T. latifolia* and *T. angustifolia* follow the same pattern. Months, sample size, location, and season exhibited substantial differences in the results. Month and Sample, Month and Location, Sample and Location, location and Season, and month x sample x location interactions with *T. latifolia* density were also significant. However, no correlation was found between the season and the density of *T. angustifolia. T. latifolia*, *T. angustifolia* densities, and phosphate-phosphorus concentration were found to have a significant correlation (P<0.05) (Tables 6, 7, 8, and 12)

Relationship between water nitrate-nitrogen concentration and *Typha* species density

nitrate-nitrogen constitutes the second most abundant element in nutrition and is present in various organic forms like carbon. Nitrogen is one of the key nutrients in the aquatic environment for the growth of *Typha* species. The results of nitrate-nitrogen water levels in the wetlands of Hadejia-Nguru and the density of *T. latifolia-T. angustif*olia are shown in Figure 5. nitrate-nitrogen

concentrations in water vary between 3mg/L and 13mg/L. *T. latifolia* was 60 to 100 stands per nine square meters and 40 to 65 stands per nine square meters, respectively. During the extreme dry season (April-May), the high value (NO_3-N) of water was obtained, while lower values were obtained during the wet season (July-August). Regarding nitrate-nitrogen concentrations, *T. latifolia* and *T. angustifolia* have the same pattern. The results revealed a significant difference (P<0.05) between months, sampling stations, locations, and seasons. Similarly, the interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* density was significant. *T. latifolia* and *T. angustifolia* density exhibited a significant correlation (P<0.05) between nitrate-nitrogen concentrations and *T. latifolia* and *T. angustifolia* densities (Tables 6, 7, 8, and 12).

Table 6. Analysis of variance of *Typha latifolia* concerning physicochemical parameters for two years (2010 and 2011)

Source of variation	DF	Water PO_4-P (mg/L)	Water NO_3-N (mg/L)	Water Mg (mg/L)
TL (Treatment)	116	0.024*	0.035*	0.036*
Month	11	0.036*	0.031*	0.042*
Sample	22	0.034*	0.330	0.012*
Location	2	0.032*	0.004*	0.008*
Season	1	0.020*	0.003*	0.011*
Month* sample	22	0.003*	0.032*	0.048*
Month* location	22	0.021*	0.002*	0.034*
Sample* location	4	0.002*	0.033*	0.026*
Location* season	2	0.040*	0.002*	0.002*
Month *sample *location	44	0.002*	0.002*	0.760
Sample location season	6	0.003*	0.043*	0.243
Error	207			

Note: *Significant; at (P<0.05), TL= *Typha latifolia*

Table 7. Analysis of variance of *Typha angustifolia* concerning physicochemical parameters concentration for two years (2010 and 2011)

Source of variation	DF	Water PO_4-P (mg/L)	Water NO_3-N (mg/L)	Water Mg (mg/L)
TA (Treatment)	116	0.434	0.020*	0.031*
Month	11	0.436	0.011*	0.002*
Sample	22	0.012*	0.312	0.001*
Location	2	0.021*	0.004*	0.004*
Season	1	0.351	0.003*	0.002*
Month* sample	22	0.028*	0.214	0.037*
Month* location	22	0.002*	0.003*	0.003*
Sample* location	4	0.027*	0.003*	0.0185
Location* season	2	0.030*	0.003*	0.013*
Month*sample*location	44	1.33	0.004*	0.019*
Sample location season	6	0.004*	0.013*	0.001*
Error	207			

Note: *significant at (P<0.05), TA= *Typha angustifolia*

Table 8. Mean values seasonal variation of surface water chemical concentrations and *Typha* species density concerning season and year in Hadejia-Nguru Wetlands

Season/ years	*T. latifolia* dens. / $1m^2$	*T. angustifolia* dens. / $1m^2$.	Water PO_4-P (mg/L)	Water NO_3-N (mg/L)	Water Mg (mg/L)
Seasons					
Wet	65.53^b	35.06^b	3.00^b	2.00^b	3.62^b
Dry	80.53^a	40.49^a	7.00^a	4.00^a	5.20^a
Years					
2010	75.91^b	50.59^b	9.13^b	5.92^b	4.56^b
2011	88.29^a	57.82^a	11.94^a	6.01^a	6.35^a

Note: Means with the same letters across columns are not significantly different (P<0.05)

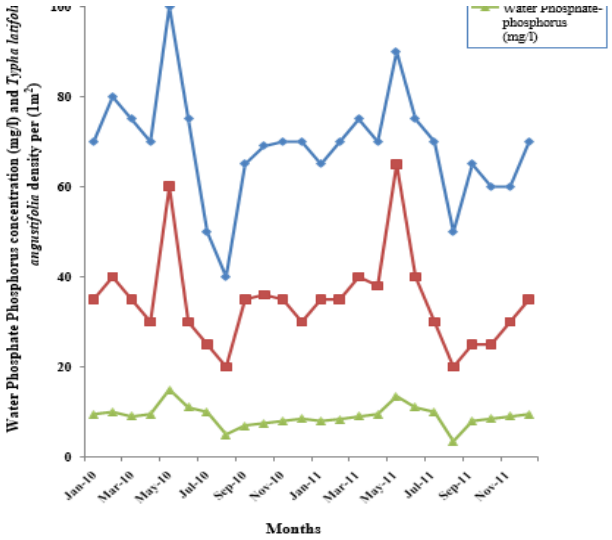

Figure 4. Monthly variation between water phosphate-phosphorus concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011.

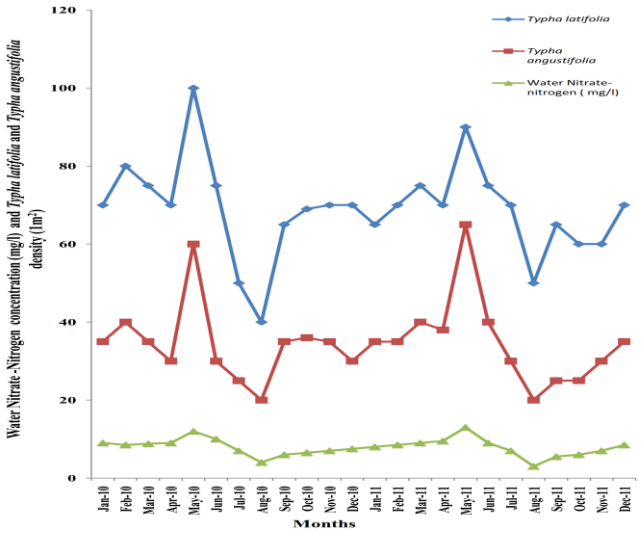

Figure 5. Monthly variation between water nitrate-nitrogen concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011.

Relationship between water Magnesium concentration and *Typha* species density

Figure 6 shows the results for water Mg concentration in Hadejia-Nguru Wetlands water. The concentration ranges from 1 to 10 milligrams per liter. *T. latifolia* density was 60 to 100 stands per $9m^{2,}$ and *T. angustifolia* density was 40 to 65 stands per $9m^2$, respectively. Water magnesium (Mg^{4-}) levels were highest during the extreme dry season (April-May) and lowest during the wet season (July-August). However, the density of *T. latifolia* and *T. angustifolia* fluctuated similarly. The results revealed a significant difference ($P < 0.05$) between months, sampling stations, location, and season. Again, the interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* density was significant. Except for month x sample x location, no significant correlation was found between magnesium concentration and *T. latifolia* density. It also revealed a significant correlation ($P<0.05$) between *T. latifolia* and the density of *T. angustifolia* and magnesium content, as well as a non-significant difference between Sample, Month, sample location (Tables 6, 7, 8, and 12).

Relationship between sediment phosphate-phosphorus concentration and *Typha* species density

Figure 7 shows the results of the soil sediment phosphate-phosphorus concentration of Hadejia-Nguru Wetlands. Phosphate-phosphorus concentrations in the silt ranged from 6.5 to 16 mg/kg. *T. latifolia* and *T. angustifolia* densities ranged from 60 to 100 stands for *T. latifolia* and 40 to 65 stands for *T. angustifolia*, respectively. During the dry season (April-May), higher sediment phosphate-phosphorus (PO_4-P) concentrations were measured, while during the peak of the rainy season, lower concentrations were recorded (July-August). *T. latifolia* and *T. angustifolia* have similar fluctuation patterns. The results also revealed that the sediment phosphate-phosphorus concentration was significant at ($P<0.05$) in all treatments, including months, sampling stations, location, and season. Similarly, there was a significant interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* and *T. angustifolia* density. It also revealed a significant correlation between the densities of *T. latifolia* and *T. angustifolia* and sediment phosphate-phosphorus concentration (Tables 9, 10, 11, and 12).

Relationship between sediment nitrate-nitrogen concentration and *Typha* species density

The content of nitrate-nitrogen in the sediment ranged from 6 to 14 mg/kg (Figure 8). *T. latifolia* and *T. angustifolia* densities were from 60 to 100 stands per $9m^3$ and 40 to 65 stands per $9m^3$, respectively. Sediment nitrate-nitrogen concentrations were greater during the extreme dry season (April-May) and lowered in the wet season (July-August). The populations of T. latifolia and T. angustifolia followed a similar trend, increasing concentrations as density rose. They examined data that revealed the sediment nitrate-nitrogen concentrations for all treatment months, sampling stations, locations, and seasons. Similarly, the interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* and *T. angustifolia* density was significant. It also revealed a significant correlation between the densities of *T. latifolia* and *T. angustifolia* and sediment nitrate-nitrogen concentrations (Tables 9, 10, 11, and 12).

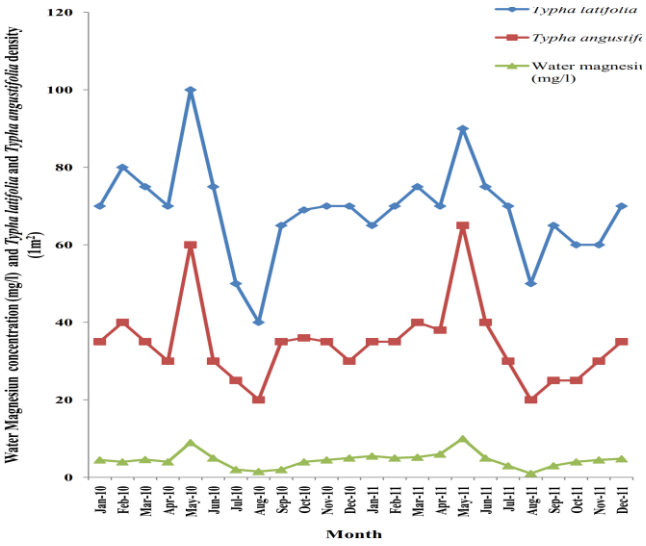

Figure 6. Monthly variation between water magnesium concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011

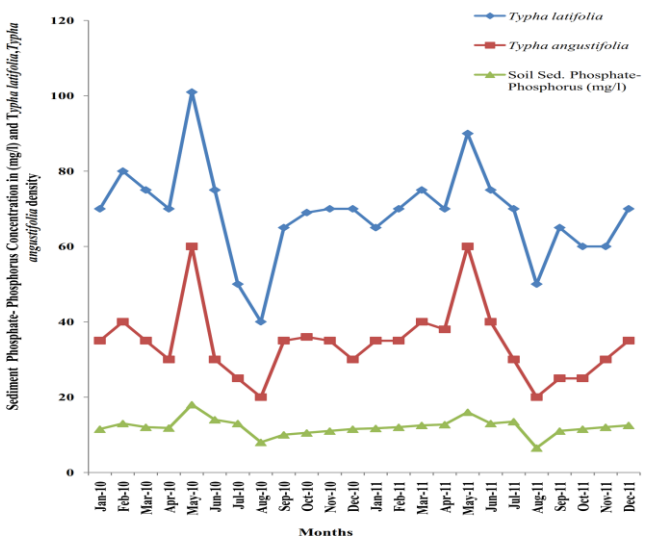

Figure 7. Monthly variation between sediment phosphate-phosphorus concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011.

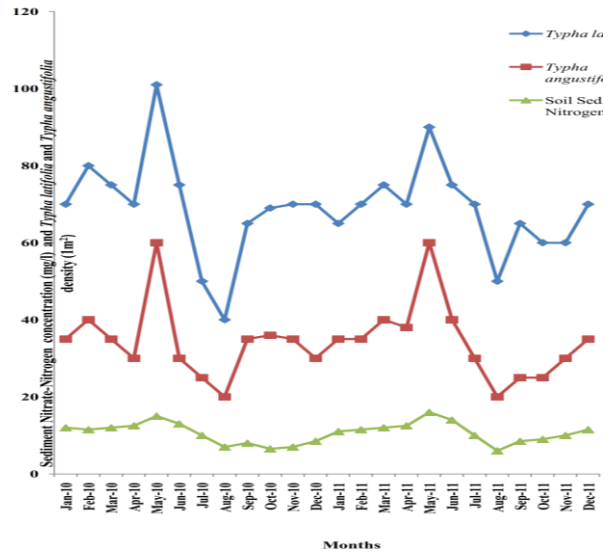

Figure 8. Monthly variation between sediment nitrate-nitrogen concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011.

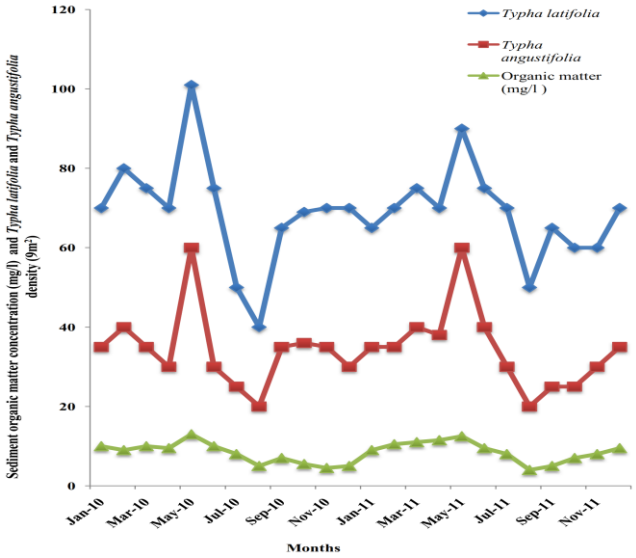

Figure 9. Monthly variation between sediment organic matter concentration and *Typha* species density in Hadejia-Nguru wetland in 2010 and 2011.

Relationship between sediment organic matter concentration and *Typha* species density

Organic matter content is the second most abundant nutrient element, and it comes in a variety of organic forms, similar to carbon. It is one of the most critical nutrients for rhizomes in *Typha* species. The organic matter concentration in the Hadejia-Nguru wetland is depicted in Figure 9. The concentration of organic materials ranged from 4 to 12 mg/kg. The density of *T. latifolia* and *T. angustifolia*, respectively, ranges from 40 to 100 and 20 to 65 stands per $9m^2$. As the wet season progressed, the amount of organic matter in the soil reduced. The maximum organic matter concentration was found during the peak of the dry season (April-May). The density of *T. latifolia* and *T. angustifolia* fluctuated in the same way. The results revealed that the concentration of sediment organic matter was considerable for all treatments, sample stations, locations, and seasons. Similarly, the interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* and *T. angustifolia* density was significant. Similarly, the interaction between month and sample, month and location, sample and location, location and season, month x sample x location, and season years and seasons with *T. latifolia* and *T. angustifolia* density was significant, except during therapy and for a few months. There was also a significant correlation ($P<0.05$) between the densities of *T. latifolia* and *T. angustifolia* and the sediment organic matter concentration (Tables 9, 10, 11, and 12).

Table 9. Analysis of variance concerning Sediment Nutrients concentration (mg/kg) for two years (2010 and 2011)

Source of variation	DF	Sediment PO_4-P (g/kg)	Sediment NO_3-N (g/kg)	Sediment OM (g/kg)
TL (Treatment)	116	0.034*	0.025 *	0.236
Month	11	0.436	0.0131*	0.000*
Sample	22	0.012*	0.0251*	0.011*
Location	2	0.021*	0.040*	0.388
Season	1	0.031*	0.028*	0.000*
Month* sample	22	0.027*	0.003*	0.238
Month* location	22	0.015*	0.032*	0.000*
Sample* location	4	0.027*	0.028*	0.226
Location* season	2	0.030*	0.028*	0.000*
Month *sample*location	44	0.033*	0.023*	0.025*
Sample location season	6	0.024*		
Error	207			

Note: *significant at ($P<0.05$), TA= *Typha latifolia*

Table 10. Analysis of variance of *Typha angustifolia* concerning sediment nutrients concentration for two years (2010 and 2011)

Source of variation	DF	Sediment PO_4-P (mg/L)	Sediment NO_3-N (g/kg)	Sediment OM (g/kg)
TL (Treatment)	116	0.334	0.031*	0.003*
Month	11	0.036*	0.023*	0.000*
Sample	22	0.001**	0.050*	0.001*
Location	2	0.003*	0.040*	0.041*
Season	1	0.031*	0.019*	0.000*
Month* sample	22	0.035*	0.001*	0.038*
Month* location	22	0.023*	0.042*	0.001*
Sample* location	4	0.027*	0.031*	0.046*
Location* season	2	0. 002*	0.032*	0.000*
Month *sample*location	44	0.023*	0.003*	0.001*
Sample location season	6	0.034*	0.015*	0.034*
Error	207			

Note: *Significant, TA= *Typha angustifolia*

Variation in water PO_4-P, NO_3-N, and Mg in different sampling stations

Chemical concentrations differ from sampling station to sampling station (Figure 10). The upper course had the highest concentration, followed by the middle and lower courses. Phosphorus, Nitrate, and Magnesium concentrations in water varied from 13 to 20 mg/L in the upper course, 12 to 16 mg/L in the middle course, and 5.5 to 12 mg/L in the lower course. From upper to lower courses, the density of *T. latifolia* and *T. angustifolia* followed the same trend in decreasing order. *T. latifolia* and *T. angustifolia* density ranges are 60 to 95 stands per $9m^2$ and 30 to 55 stands per $9m^2$, respectively. With *T. latifolia* and *T. angustifolia* densities, there was a substantial variation in water PO_4-P, NO_3-N, and Mg concentrations. The density of these aquatic plants grew as the chemical concentration increased (Table 13 and 14). From upper to lower courses, the density of *T. latifolia* and *T. angustifolia* followed the same pattern in decreasing order. *T. latifolia* and *T. angustifolia* density ranges are 60 to 95 stands per $9m^2$ and 30 to 55 stands per $9m^2$, respectively. With *T. latifolia* and *T. angustifolia* densities, there was a substantial variation in water PO_4-P, NO_3-N, and Mg concentrations. The density of these aquatic plants grew as the chemical concentration increased (Table 13 and 14).

Variation in sediment PO_4-P, NO_3-N, and organic matter in different samplings stations

Chemical concentrations differ from sample station to sampling station (Figure 11). The upper course had the highest concentrations, followed by the middle and lower courses. Sediment phosphate-phosphorus, nitrate-nitrogen, and organic matter concentrations in the upper, middle, and lower course varied from 23 to 35 mg/kg, 16 to 22 mg/kg, and 13 to 20 mg/kg, respectively. The density of *T. latifolia* and *T. angustifolia* decreased in the same sequence from upper to lower course. *T. latifolia*, *Typha*, and *T. angustifolia* density ranged from 60 to 100 stands, 30 to 55 stands, and 30 to 55 stands per $9m^2$, respectively. Water PO_4-P, NO_3-N, and organic matter concentrations differed significantly ($P<0.05$) with *T. latifolia* and *T. angustifolia* densities of aquatic plants increased as chemical concentrations increased (Tables 13 and 14).

Impact of *Typha* species on fish catch and distribution in Hadejia-Nguru wetland (2010 and 2011)

Tables 15 and 16 show that the average water, air temperature, dissolved oxygen (DO), Biological oxygen demand (BOD), number, and weight of fish caught monthly for two years in A (un-infested area) ranged from 795 to 1.948, 83,167.9 to 173,026.8kg and B (*Typha* infested area) 150 to 600, 14,402 to 59,355 in Hadejia-Nguru Wetlands. In 2011, the number of fish species and their weight declined. In A (open water area), twenty-three different families and sixty-two fish species were caught in 2010, while twenty-two families and fifty-six species were seen in 2011. As a result of the increase in *Typha* species infestation, one family and eight species were lost in 2011. The results were statistically analyzed for both 2010 and 2011, revealing a substantial difference between the months. There was a significant difference of (DO) and (BOD) significant infested areas. The air temperature did not differ significantly between (January, February, and March) (May and June), (August and September) (November and December). The number and weight of fish taken in two locations in both years showed a similar pattern (Tables 15 and 16). There was a significant difference in the weights and number of fish taken in A (*Typha* uninfested area) and B (*Typha* infested area) between seasons and years (Table 17).

Table 11. Mean values (g/kg) for some sediment PO_4-P, NO_3-N, and Organic matter between seasons and years in Hadejia-Nguru wetland

Season/ years	*T. latifolia* dens. / $1m^2$	*T. angustifolia* dens. / $1m^2$.	Sediment PO_4-P (g/kg)	Sediment NO_3.N (g/kg)	Sediment OM (%)
Seasons					
Wet	70.53^b	30.06^b	4.10^b	2.50^b	5.62^b
Dry	95.53^a	45.49^b	7.50^b	4.50^a	8.80^a
Years					
2010	80.01^b	60.09^b	13.23^b	8.52^b	7.56^b
2011	90.09^a	65.02^a	15.94^a	10.02^a	8.05^a

Note: Means with the same letters across columns are not significantly different at ($P<0.05$)

Table 12. Correlation between *Typha latifolia*, *T. angustifolia,* and physicochemical parameters and soil nutrients

Variables	TL	TA	Water (PO_4P) mg/L	Water (NO_3N) mg/L	Water (Mg^4) mg/L	Sediment (PO_4P) g/kg	Sediment (NO_3N) g/kg	Sediment Org. matter g/kg
TL	1.000							
TA	0.0031	1.000						
Water (PO_4P)	0.032*	0.002*	1.000					
Water (NO_3N)	0.0234*	0.0203*	0.010*	1.000				
Water (Mg^4)	0.0245*	0.0140*	0.0412*	0.0101*	1.000			
Sed. (PO_4P)	0.0341*	0.0234*	0.0341*	0.0162*	0.0301*	1.000		
Sed. (NO_3N)	0.0001*	0.0210*	0.0323*	0.0231*	0.0134*	0.0231*	1.000	
Sed. Organic matter	0.0020*	0.3102*	0.023**	0.102**	0.0010**	0.2312**	0.2103**	1.000

Note: *Significant at $P<0.05$; **Significant at $P<0.01$, TL: *Typha latifolia,* TA: *Typha angustifolia,* Sed. : Sediment

Management methods

Figure 11 shows biological management in the Hadejia-Nguru Wetlands using different *P. karka* weights. There were few changes in the first five months. However, as the weight of *P. karka* increased, the density of *Typha* species reduced. As demonstrated in Figure 12, the control experiment, free of *P. karka*, continued to grow each month. The best controls were obtained between June and August and can be linked to low physicochemical parameters and sediment nutrients. The experiment also revealed that the more *P. karka* weight, the more effective the control. The data analysis revealed that all treatments, years, and seasons had significant differences. However, there was no significant difference between the control experiment (Tables 18 and 19).

Manual control of Typha latifolia *and* T. angustifolia *using cutting*

The manual control results showed that in February, April, and June, the *Typha* species cut at 5cm, 10cm, and 15cm below the water showed no more re-growth (Figure 13). It was also discovered that the lower the incision below the water, the better the control of *Typha* species was. At the same time, the density of *Typha* species cut at 5cm, 10cm, and 15cm above the water (which served as a control experiment) continued to rise. These also demonstrated a consistent rate of re-growth. There were significant differences at ($P<0.05$) between all cutting levels, years, and seasons, according to the results (Tables 20 and 21). Above the water, however, there was no significant difference in cutting levels.

Physical control of Typha latifolia and T. angustifolia using shading

Figure 14 shows the results of the impact of shade on *Typha* species. The results showed that when triple black tarpaulin was utilized, *Typha* species only lasted four months, while doubled tarpaulin lasted eight months, and single tarpaulin lasted thirteen months. The shade's efficiency was determined by the shading effect of the black tarpaulin cover. After two months, the control experiment with the single, double, and triple black tarpaulin and control increased. The findings also demonstrated that the same black tarpaulin used to control *Typha* species could manage other aquatic macrophytes. The data analysis showed significant differences between single, double, triple, year, and seasons (Tables 22 and 23). Different techniques of controlling *T. latifolia* and *T. angustifolia* have their mean values compared. *Typha* mortality was highest when cutting below the water, followed by shading with black tarpaulin and a biological approach utilizing P. karka (Table 24).

Discussion

Typha species resident in Hadejia-Nguru Wetland

Using an aquatic plant information system, two species of *Typha* were found during the two years of research. *T. latifolia* and *T. angustifolia* were the species in question, with infection levels of 65% to 70% and 30% to 35%, respectively. This contradicts Wetzel's study (2003) that *T. angustifolia* is not abundant in West African wetlands. Their densities ranged from 60 to 100 stands per $9m^2$ and 40 to 60 stands per $9m^2$, respectively. *T. latifolia* has a larger density than *T. angustifolia* due to seasonal changes caused by flooding (flood fluctuation). This supports Smith's (2004) results that *T. latifolia* can endure a wider variety of tropical environments than *T. angustifolia. T. angustifolia*, on the other hand, tended to grow slightly in density during higher floods. This could be related to the fact that it could withstand higher flood levels. Environmental variables may play a significant difference between *T. latifolia* and *T. angustifolia*. Wilcox (2006) made a similar observation at New Delhi Lake, where phosphate-phosphorus levels rose due to irrigation efforts, increasing the prevalence of *T. latifolia* and eradicating practically all other aquatic macrophytes.

Table 13. Mean values of chemical parameters of surface water in sampling sites and *Typha latifolia, T. angustifolia* density in Hadejia-Nguru Wetlands

Sample	*Typha latifolia* density per $9m^2$	*Typha angustifolia* density per $9m^2$	Water $(PO\text{-}P)_4$ (mg/L)	Water $(NO\text{-}N)_3$ (mg/L)	Water2+ Mg (mg/L)
Upper course	74.620^a	27.953^a	1.494^a	0.702^a	0.598^a
Middle course	73.722^b	25.521^b	1.362^b	0.601^b	0.384^b
Lower course	71.435^c	23.721^c	0.800^c	0.502^c	0.280^c

Table 14. Mean values of sediment nutrients in sampling sites and *Typha latifolia, T. angustifolia* density in Hadejia-Nguru Wetlands

Samples	*Typha latifolia* density per $9m^2$	*Typha angustifolia* density per $9m^2$	Sediment $(PO\text{-}P)^4$ (g/kg)	Sediment $(NO\text{-}N)^3$ (g/kg)	Sediment organic matter (%)
Upper course	74.620^a	27.953^a	3.494^a	2.002^a	2.008^a
Middle course	73.722^b	25.521^b	$b2.262^b$	1.201^b	1.840^b
Lower course	71.435^c	23.721^c	$c1.100^c$	0.702^c	0.600^c

Table 15. The average (DO), (BOD) Air temperature, Number, and weight of fish caught in two locations, A (Un-infested) and B (*Typha* species infested area) in Hadejia-Nguru wetland 2010.

Month	Family name	Species	Area A number	Weight (kg)	DO (A) mg/L	BOD (A) Mg/L	Area B number	Weight kg	DO (B) 9mg/L	BOD (B) mg/L
Jan 2010	Dasytidae, Protopteridae, Polypteridae	*Dasyatis garouaensis, Protopterus annectens, Polypterus ansorgi, Polypterus bichirbihir, Polypterus* (e) *endicheri, Polypterus(s) senegalus, Erpetoiochthys calabaricus*	842	86,187	5.9	15	162	17,400	4.4	14..5
Feb 2010	Clupeidae	*Odaxothris samento, Pellonula vorax*	1,227	127, 200	6.8	15.5	225	39,460	4.5	14.1
	Osteoglosidae, Pantodontidae	*Heterotis niloticus, Pantodon buchholzi*								
	Mormyridae	*Mormyrus rume, Mormyrus tapiru,s Hippopotamyrus psittacu,s Mormyrops anguilloides, Campylomyrustaman dua*								
Mar 2010	Gymnarchidae	*Marcuseninus syprinoides, Marcuseninus senegalensis, Gytmnorchus niloticus*	1,051	134,809	7.5	15.1	250	30,047	5.0	14.0
	Cromeridae, Hepsetidae	*Cromeia nilotica, Hepsetusodoe*								
	Characidae	*Hydrocynus brevis, Hydrocynus vittatus, Hydrocynus forskalii, Alestes dentex, Brycinus leuciscus, Micralestes elongates*								
Apr 2010	Distichodontidae	*Phago loricatus*	111	170,142	6.8	15.8	200	37,057	4.2	13.5
May 2010	Citharinidae	*Distichodusengyce phajus, Citharidiumansorgii, Citharinus latus, Citharinus citharinus*	1,150	154,929	6.2	15.3	310	40,015	4.0	14.2
Jun 2010	Cyprinidae, Icthyboridae	*Chelaethiops bibie, Labeo coubie, Labeo senegalensis, Barbus bynni occidentalis*	1,948	140,147	6.8	15.4	400	34,500	4.0	14.2
Jul 2010	Bagridae	*Bagrus docmak, Bagrus filamentosus, Bagrus bajad, Clarotes laticeps, Chrysichthys aluluensis, Chrysichthys auratus, Chrysichthys nigrodigitatus, Auchenoglanis biscutatus*	1,334	133,026	7.0	16.0	361	23,633	4.1	14.6
Aug 2010	Schilbeidae	*Parailia pellucid, Schilbe intermedius, Schilbe mystus*	1,025	127,315	9.0	15.8	467	25,236	4.2	14.8
Sep 2010	Clariidae	*Gymnalla bestypus, Heterobranchus isopterus, Clarias gariepinus, Clarias anguillaris, Clarias jaensis, Clarias macromystax*	1,063	119,773	8.4	15.0	474	35,910	4.7	14.6
	Malapteruridae	*Malapterurus electricus, Malapterurus minjiriya*								
Oct 2010	Channidae	*Parachanna africana, Parachanna obscura*	1,070	114,473	7.4	15,9	329	48,137	46	14,7
	Mochokidae	*Chiloglanis benuensis, Synodontis resupinatus, Synodontis budgetti, Synodontis clarias , Synodontis omias, Synodontis robbianus, Synodontis nigrita, Synodontis schitt*								
Nov 2010	Channidae	*Parachanna african Parachanna obscura*	1,047	115,656	8.2	16,1	402	53,338	4,8	14,3
	Centropodae	*Lates niloticus, Chromidatilapia guntheri, Tilapia dageti, Tilapia zilli, Tilapia guineensis*								
Dec 2010	Channidae	*Parachanna african Parachanna obscura*	1,000	115,656	8.2	16,1	402	53,338	4,3	14,3
	Centropomidae	*Lates niloticus, Chromidotilapia guntheri, Tilapia dageti, Tilapia zilli, Tilapia guineensis*								

Table 16. The average (DO), (BOD) Air temperature, Number and weight of fish caught in two locations, A (Un-infested) and B (*Typha* species infested area) in Hadejia-Nguru wetland 2011

Month	Family name	Species	Area A number	Weight (kg)	DO (A) mg/L	BOD (A) Mg/L	Area B number	Weight kg	DO (B) 9mg/L	BOD (B) mg/L
Jan 2011	Protopteridae Polypteridae	*Protopterus annectens* *Polypterus ansorgii, Polypterus bichir, Polypterus endltcheri, Erpetoiochthys calabaricus*	800	83,167	6.0	15,4	127	14,402	4,2	14,5
Feb 2011	Clupeidae, Osteoglossidae, Pantodontidae, Mormyridae	*Odoxothris samento, Heterotis niloticus, Pantodon buchholzi* *Mormyrus rume, Mormyrus tapirus, Hippopotamyrus psittacus, Campylomyrus tamandua, Marcuseninus syprinoides*	1,100	117,199	5.9	16,1	600	31,465	5,0	14,2
Mar 2011	Gymnarchidae, Cromeridae, Hepsetidae, Characidae	*Gytmnorchus niloticus, Cromeia nilotica, Hepsetidae odoe, Hydrocynus brevis, Hydrocynus vittatus, Hydrocynus forskalii, Alestes dentex, Brycinus leuciscus, Micralestes elongates*	1,022	121,799	6.7	15,3	250	30,047	4,6	14,4
Apr 2011	Distichodontidae	*Phagolo ricatus Distichodusengy cephajus*	111	170,142	6.9	15,5	150	32,067	4,7	14,4
May 2011	Citharinidae	*Citharidium ansorgii, Citharinus latus, Citharinus citharinus Chelaethiops bibie, Labeo coubie*	1,150	134,929	7.2	15,3	300	40,000	4,3	14.9
Jun 2011	Cyprinidae	*Chelaethiops bibie, Barbus occidentalis*	1,338	121,472	8.7	15,6	403	25,510	4,3	14,6
Jul 2011	Bagridae	*Bagrus docmak, Bagrus filamentosus, Bagrus bajad, Chrysichthys aluluensis Auchenoglanis biscutatus*	1,334	173,026.8	8.4	15.4	355	43,643	4.1	14.8
Aug 2011	Schilbeidae	*Paraphilia pellucida, Schilbe intermedius, Schilbe mystus*	795	11,305	7.8	15.6	450	42,365	4.0	14.6
Sep 2011	Clariidae	*Gymnalla bestypus, Heterobranchus isopterus, Clarias gariepinus, Clarias anguillaris, Clarias macromystax, Malapterurus electricus*	953	109,763	6.9	15.4	479	57,714	4.2	14.8
Oct 2011	Malapteruridae Mochokidae	*Malapterurus minjiriya* *Chiloglanis benuensis, Synodontis resupinatus, Synodontis budgetti*	1,030	142,463	5.0	15.4	370	38,147	4.6	14.5
Nov 2011	Channidae Centropmidae	*Parachanna africana Parachanna obscura* *Lates niloticus, Chromidotilapia gunther, Tilapia dageti, Tilapia zillii*	1,000	111,646	6.8	15.5	450	41,348	5.0	14.2
Dec 2011	Anabantidae	*Clenopoma nebulosum, Ctenopoma murei, Ctenopoma paetherici*	1,043	102,346	7.5	15.4	430	48,233	4.9	15.0

Table 17. Mean values per ($1m^2$) of fish caught in *Typha* species free and *Typha* infested area between the seasons and years in Hadejia-Nguru Wetlands

Source of variation	Mean values
(A) *Typha* unifested area	85.998[a]
(B) *Typha* infested area	60.880[b]
Seasons	
Dry (A) *Typha* unifested area	90.324[a]
Dry (B) *Typha* infested areas	40.562[b]
Wet (A)*Typha* Unifested area	50.231[a]
Years	
2010 (A)*Typha* unifested area	85.761[a]
2010 (B) *Typha* infested areas	55.264[b]
2011 (A) *Typha* unifested area	70.452[a]
2011 (B) *Typha* infested areas	42.642[b]

Note: Means with the same letters across column are not significantly different at (P<0.05)

Table 18. Mean values of difference *Phragmites karka* and mortality rate per ($1m^2$) of *Typha latifolia* and *T. angustifolia* interplanted with different *P. karka* weight Hadejia Nguru Wetlands

Treatments	Mean value of mortality rate of *Typha latifolia, T. angustifolia*
50kg of *Phragmites karka*	55.342[a]
25kg of *P. karka*	33.653[b]
10kg of *P. karka*	25.672[c]
Free from *P. karka*	10.324[d]

Note: Means with the same letters across columns are not significantly different at P<0.05

Table 19. Mean values of different *Phragmites karka* and mortality rate per ($1m^2$) of *Typha latifolia* and *Typha angustifolia* interplanted with different *Phragmites karka* weight concerning years and seasons in Hadejia Nguru Wetland.

Source of variation	50kg of *P.karka*	25kg of *P.karka*	10kg of *P.karka*	Free from *P.karka*
Year				
2010	90.232[b]	53.127[b]	20.653[b]	345.230[a]
2011	120.620[a]	75.230[a]	32.234[a]	345.231[a]
Seasons				
Dry	50.342[b]	42.103[b]	25.120[b]	246.781[a]
Wet	75.531[a]	55.107[a]	35.708[a]	246.781[a]

Note: Means with the same letters across columns are not significantly different at P<0.05

The initial *Typha* species community arose due to multiple succession routes or colonization from nearby water and other physical characteristics that allowed for water or wind dissemination or direct transportation by waterfowls. Due to seasonal and periodic changes, changes in the environment may also impact *Typha* species (Chiroma et al., 2005). The distribution, composition, and quantity of *T. latifolia* and *T. angustifolia* in the Hadejia Nguru wetlands could be linked to various events, including the construction of the Tiga dam upstream, which changed the environmental circumstances.

Table 20. Mean values of different cutting levels of *Typha latifolia* and *T. angustifolia* and re-growth rate per ($1m^2$) in Hadejia Nguru Wetlands

Treatments	Mean value re-growth rate of *Typha latifolia, T. angustifolia (*$1m^2$**)**
15 cm above the water	95.342[a]
10cm above the water	80.321[b]
5cm above the water	72.461[c]
5cm below the water	55.672[d]
10cm below the water	33.653[e]
15 cm below the water	15.342[f]

Note: Means with the same letters across columns are not significantly different at P<0.05

Table 22. Mean values of difference black tarpaulin number and mortality rate per ($1m^2$) of *Typha latifolia* and *T. angustifolia* covered with black tarpaulin Hadejia Nguru Wetlands

Treatments	Mean value re-growth rate of *Typha latifolia, T. angustifolia (*$1m^2$**)**
Triples black tarpaulin	80.342[a]
Double black tarpaulin	40.653[b]
Single black tarpaulin	21.672[c]
Without black tarpaulin	8.324[d]

Note: Means with the same letters across columns are not significantly different at P<0.05

Table 23. Mean values of different numbers black tarpaulin and *Typha latifolia-T. angustifolia* mortality rate per (1m2) concerning years and seasons in Hadejia-Nguru wetland

Source of variation	Without black tarpaulin	Single black Tarpaulin	Double black tarpaulin	Triples black tarpaulin
Year				
2010	70.451[a]	63.000[a]	60.345[a]	45.306[a]
2011	70.443[a]	52.125[b]	50.798[b]	35.202[b]
Seasons				
Dry	69.532[a]	58.324[a]	35.307[a]	35.812[a]
Wet	68.435[a]	45.453[b]	25.209[b]	20/254[b]

Note: Means with the same letters across columns are not significantly different at P<0.05

Table 24. Comparison of mean values of different peak levels of mortality rate per ($1m^2$) of *Typha latifolia* and *T. angustifolia* with different methods of controlling in Hadejia-Nguru wetland

Methods	Mean value of mortality rate of *Typha latifolia, T. angustifolia*
Manual control (Cutting below the water levels)	95.653[a]
Mechanical control (Shading using black tarpaulin)	54.753[b]
Biological control (*Phragmites karka*)	25.765[c]

Note: Means with the same letters across columns are not significantly different at (P<0.05)

Table 21. Mean values of different cutting levels of *Typha latifolia* and *T. angustifolia* re-growth rate per ($1m^2$) concerning years and seasons in Hadejia-Nguru Wetlands

Source of variation	15 cm above the water	10 cm above the water	5cm above the water	15 cm below the water	10cm below the water	5cm below t water
Year						
2010	98.673[a]	65.342[a]	50.875[a]	20.365[a]	35.642[a]	45.678[a]
2011	98.672[a]	65.456[a]	50.798[a]	10.123[b]	30.750[b]	40.320[b]
Seasons						
Dry	98.654[a]	65.342[a]	50.860[a]	15.646[a]	33.123[a]	35.105[a]
Wet	98.653[a]	65.453[a]	50.873[a]	6.532[b]	25.432[b]	30.000[b]

Note: Means with the same letters across columns are not significantly different at ($P<0.05$)

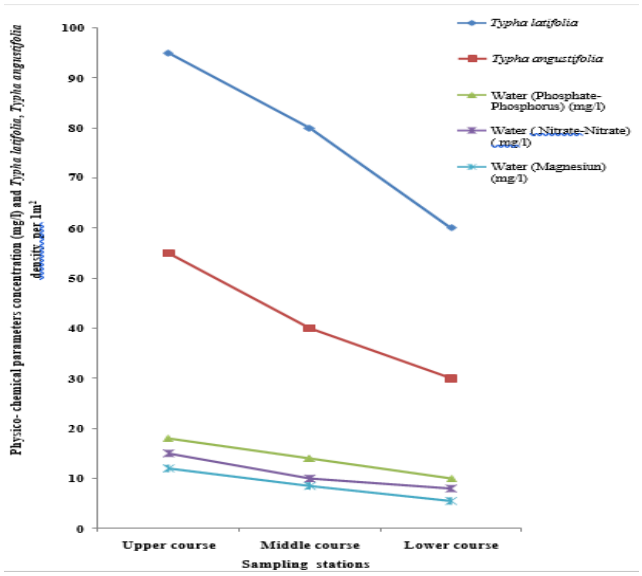

Figure 10. Variation between surface water PO_4-P, NO_3-N, and Mg concentrations in different sampling stations and *Typha* species density in Hadejia-Nguru wetland

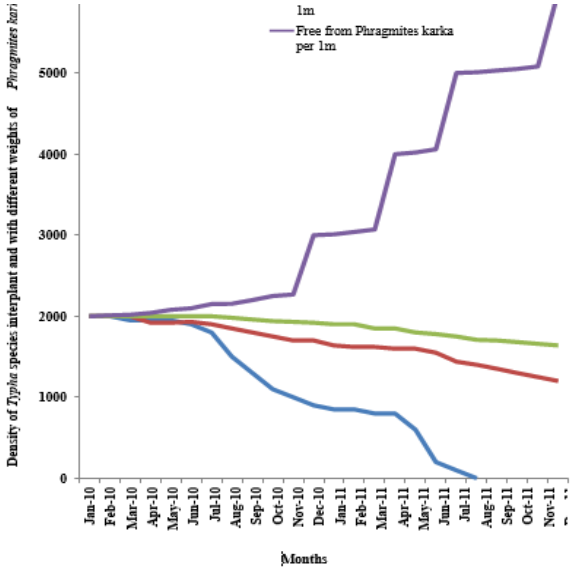

Figure 12. Monthly variation between different *Phagramites karka* weight and *Typha latifolia* and *T. angustifolia* cut at different levels in Hadejia-Nguru, wetland (2010 and 2011)

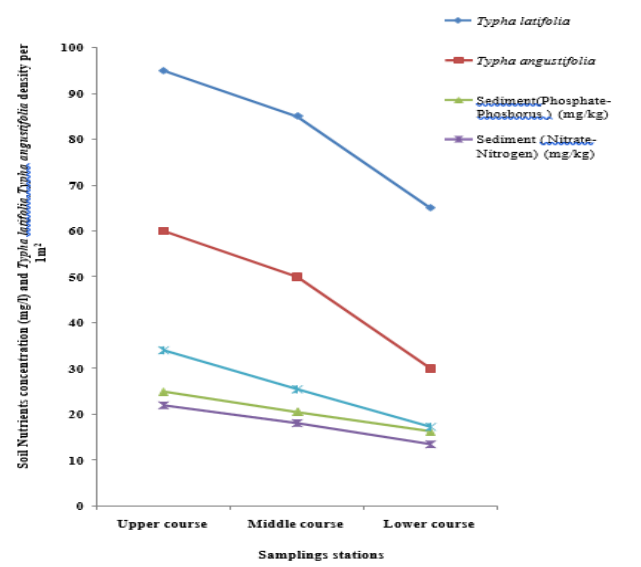

Figure 11. Variation between sediment PO_4-P, NO_3-N, and sediment organic matter in different sampling stations and *Typha species* density in Hadejia-Nguru Wetlands

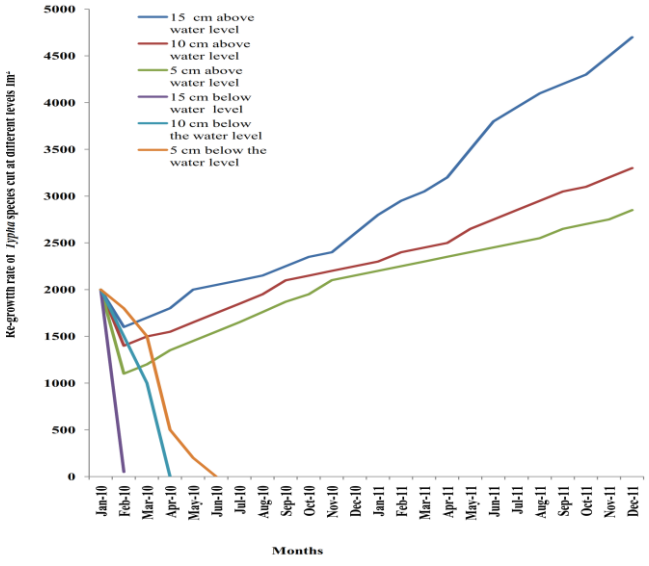

Figure 13. Monthly variation between different regrowth rates of *Typha latifolia* and *T. angustifolia* density in Hadejia-Nguru wetland in the years 2010 and 2011

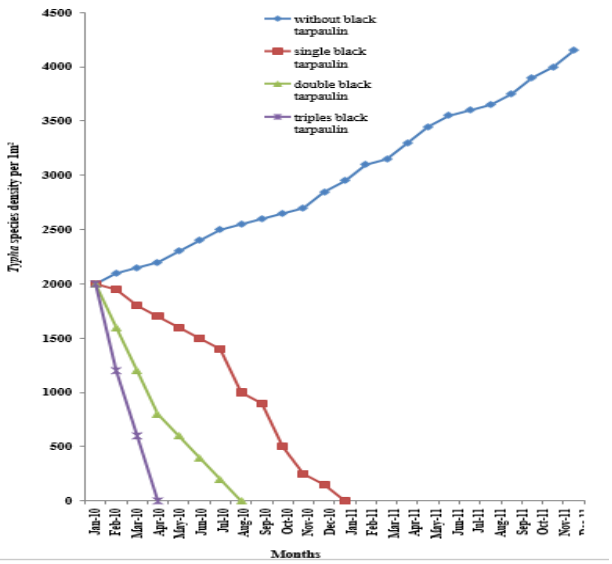

Figure 14. Monthly variation between different numbers of black tarpaulin number and *Typha latifolia-T. angustifolia* density in Hadejia-Nguru wetland in the year 2010 and 2011

According to Wetzel (2003), environmental conditions change favor aquatic macrophytes infestation. The complicated link connects ecosystem components to the succession pattern via biotic and abiotic factors. The cycling of materials from non-living abiotic sources through producers, consumers, and decomposers is one of these interconnected elements. Smith (2009) discovered a strong correlation between the nitrogen cycle and the *Typha* species structure on the one hand. He found that increasing water and sediment nutrients boosted the density of *Typha* species. Climate, rainfall patterns, water usage, and human activities all impact water chemistry and *Typha* species. The major water chemistry and *T. latifolia, T. angustifolia* differences in densities in Hadejia Nguru wetland could have been accounted for by the extent of human activities based on locations, catchment characteristics, and water volume fluctuations, which vary from time to time as a result of seasonal variations. Smith (2009) found that water chemistry impacted the density of *T. latifolia* and *Typha diminigensis*. The water levels in the Hadejia Nguru wetland fluctuated throughout the seasons, with the highest volume during the rainy season. When the water level drops to around one-third of its greatest capacity during the dry season, it shows the most variance. As the dry season progressed, the entire littoral area, covered during the wet season, became exposed. Increased anthropogenic activities, such as increased water demand through intense irrigation around the surrounding wetlands, block making, washing, and cattle population concentration, could be linked to the overall alteration in water chemistry. As the draw-down continued, the vegetation of the exposed littoral area also increased—the rate of livestock droppings and urine increased due to grazing activity. As the wet season begins, all of these exposed locations become inundated, resulting in a build-up of organic debris. These variances could be attributable to differences in individual characteristics of the Hadejia Nguru wetlands in response to seasonal variations in water physicochemical properties, influencing the densities of *Typha latifolia* and *T. angustifolia*.

The morphology of the Hadejia Nguru wetland basin has a significant impact on physical, chemical, and biological parameters that may all be used to estimate the value of wetlands (Singh et al., 2006). *Typha* species react in varied ways to changes in their drainage basins throughout time due to biological factors and the usage of wetland categorization. Stone (2001) found that the ecological connections between *Typha* species had changed significantly. Gradual drying and shrinking of the Hadejia Nguru wetland surface area resulted in enhanced proliferation of *T. latifolia* and *T. angustifolia*. Individual adaption patterns, survival, life history patterns, and generation lines may have contributed to the development of *T. latifolia* and *T. angustifolia* in the Hadejia Nguru wetland. The extent and nature of the Sudan savannah's irregular and harsh environmental conditions, particularly climatic variations caused by wind, may impact the wetland environment (Smith 2004).

In the Hadejia Nguru wetlands, the distribution of *T. latifolia* and *T. angustifolia* was higher in the upper, middle, and lower courses. This may be due to the fact that human activities in the Hadejia Nguru wetland are concentrated in the upper and middle portions, with virtually very few activities in the lower course. The quantity of water nutrients and sediment nutrients altered the character and distribution of *Typha* species. The differences in the high density of *Typha* species demonstrate this. Changes in organic matter accumulation and ecosystem stability may also influence the makeup of *Typha* species, as seen by the higher population density of species in the upper and middle courses. The extent and size of the catchment regions, soil qualities, topography, and vegetation cover could all be connected to the wetland's primary physicochemical parameters. The intensity and rate of human activities could have considerably affected the Hadejia Nguru wetland due to its bigger catchment size and population. Irrigation, fishing, and farming along coastal borders and in catchment areas may have contributed to the increasing prevalence of *Typha* species in the Hadejia Nguru wetland.

Surface water, PO_4-P, NO_3-N, and Mg concentrations

In the Hadejia Nguru wetlands, high transparency and conductivity turbidity did not affect the density of *T. latifolia* and *T. angustifolia*. This could be due to the non-response of *Typha* species to specific physicochemical factors. Singh et al. (2006) made a similar observation. *Typha* species density was unaffected by transparency or turbidity, which could be linked to the ability of *Typha* species to tolerate different climatic conditions and environmental changes. This extremely fast-growing plant is sometimes considered an invasive native in aquatic ecosystems (Murkin and Ward 1980). Higher or lower pH did not affect the density of *T. latifolia* and *T. angustifolia* in Hadejia Nguru wetlands, which could be due to the ability of *Typha* species rhizomes to minimize higher pH

concentration. This is consistent with the findings of Singh et al. (2006) that *Typha* species can tolerate a wide range of pH because the rhizome has the potential to reduce higher pH to a lower one. However, this contradicts the findings of Balarabe (2001), who found that decreased pH resulted in a reduced density of aquatic macrophytes. Plants that use dissolved oxygen (DO) do not need oxygen and even give it out during photosynthetic activities. Because plants produce oxygen into the atmosphere, areas with dense *Typha* species have plenty of oxygen. Comparing areas with high *Typha domingesis* is growth to areas lacking *T. domingesis* is growth, Smith (2009) found that areas with high *T. domingesis* growth had greater oxygen. The density of *T. latifolia* and *T. angustifolia* is influenced by Biological Oxygen Demand (BOD). BOD was required for microorganisms to decompose organic materials. This is consistent with the findings of Smith (2009) that low BOD had a minor impact on *T. latifolia* density due to the sluggish decomposition of organic waste. Based on watershed parameters, the level of human activities influences the water chemistry of the Hadejia-Nguru wetland. Water volume changes accounted for significant limnological discrepancies between T. latifolia and T. angustifolia densities, varying from time to time due to seasonal variation. According to Torres-Orozeo et al. (1996), environmental factors may have influenced variance in lakes in New Delhi, India. The water level of Hadejia-Nguru Wetlands changed throughout both seasons, with the wet season having the highest volume. When the water level declined significantly during the dry season, the wetland showed more variety (NIFFR 2002).

The substantial influence of nitrate-nitrogen (NO3-N), phosphate-phosphorus (PO4-P), and organic matter concentrations could have been attributed to human activities such as livestock grazing, watering, and irrigation within the extended catchment and coastal areas of Hadejia-Nguru wetland. This could be associated with greater fecal matter deposition along the wetland's periphery. Nitrate-nitrogen (NO3-N) content may be predicted to grow dramatically under the impact of edaphic variables, notably during flood seasons and organic pollution (Stone 2001). Metabolic processes in the water body determine the amount of nitrate in solution at any specific time through deposition and decomposition (Smith 2004). Continuous mixing and resuspension of sediment particles may have influenced *T. latifolia* and *T. angustifolia* densities by increasing nutrient cycling and release in water. The influence of transparency and electrical conductivity on the growth of *T. domigesis* and *T. latifolia* was studied under higher and lower transparency and electrical conductivity, according to Habibah et al. (2012). There was no evidence of a link between *Typha dominingsis* and *T. latifolia*. This is consistent with the findings of this research.

Phosphorus concentrations were generally higher in the upper stream. This could be linked to the agricultural activities that take place in the area, which include both irrigation and rain-fed crops. The concentration of phosphate-phosphorus at all sites followed a similar pattern, with Phosphorus levels rising as the dry season progressed. The concentration was maximum near the end of the dry season (April-May). During this time, *T. latifolia* and *T. angustifolia* had the largest numbers. This could be because both species require phosphorus at all phases of growth. This verifies Smith's (2004) results that phosphate-phosphorus is essential for rhizome phloem and xylem production, as well as flowering.

Nitrate-nitrogen is the second most abundant nutritional element, and it appears in a variety of organic forms, similar to carbon. Nitrogen is a crucial ingredient for the growth of *Typha* species in the aquatic environment. The amount of nitrate-nitrogen at all sampling sites followed a similar pattern during the study period, with concentrations increasing as the dry season proceeded. The maximum concentration was found right after the rainy season (April-May). The top course yielded the highest concentration. This could be linked to run-off water and agricultural activity, which could explain why there are more *T. latifolia* and *T. angustifolia* plants at this location. The findings revealed a substantial variation in nitrate-nitrogen concentration between *T. latifolia* and *T. angustifolia.* Magnesium levels were found to strongly correlate with the abundance of *T. latifolia* and *T. angustifolia.* During the extreme dry season (April-May), high values of water magnesium (Mg2+) were measured, with lower values obtained during the wet season (July-August). *T. latifolia* and *T. angustifolia* have the same fluctuating pattern. The increase and decrease in water volume due to seasonal change could explain these variances in water chemical fluctuations (wet and dry season). Hillman (2012) observed that repeated filling and slow drying frequently culminate in total dehydration, resulting in vast variations in aquatic habitats' physical and chemical characteristics. The results revealed a significant difference in water Magnesium concentration and *T. latifolia*, *T. angustifolia*, except within month and sample site, at ($P<0.05$). Sediment PO4-P, NO_3-N, organic matter concentration and *T. latifolia, T. angustifolia* density

During the extreme dry season (April-May), high dry season means phosphate-phosphorus (PO4-P) levels were recorded, while lower values were observed between July and August. The results also revealed a substantial variation in the concentration of Sediment phosphate-phosphorus and *T. latifolia* and *T. angustifolia*. This could be due to the increased concentration of surface run-offs, which carried in plant materials and debris, which degraded and delivered nutrients to the soil throughout the dry season. Similarly, Green (2013) found that sediment phosphate-phosphorus, which is highly soluble in water and does not attach to the soil, has a strong migratory potential through the soil when water volumes decrease, resulting in high concentrations.

Nitrate-nitrogen in Sediment is the second most prevalent nutritional element, and as carbon, it can be found in various organic forms in soil nitrate. In the aquatic environment, nitrogen is a key nutrient for the growth of *Typha* species. During the severe high dry season (April-May), the mean value of nitrate-nitrogen (NO_3-N) was obtained. The lowest results were acquired within July to August. Both *Typha* species have the same fluctuating

pattern. These results could be related to the species' reaction to increased nitrate-nitrogen levels. This verified the effect of Birnin-Yauri et al.'s (2004) study that a rise in nitrate-nitrogen increased *Typha australis* density. Organic matter concentration is the second most abundant nutrient element, and it comes in a variety of organic forms, similar to carbon. During the study period, the maximum concentration of sediment organic matter content was found in Hadejia-Nguru Wetlands during the extreme dry season (April-May). The lowest concentration was seen during the peak of the rainy season (July-August). Both *T. latifolia* and *T. angustifolia* grew in a similar pattern of organic matter. The results revealed a substantial difference in the concentrations of Sediment Organic Matter. These could be linked to all run-off detritus ending up in sediment. Green (2013) found that both soil and water organic matter concentrations were highly soluble in water, did not attach to the soil, had a high migratory potential across the soil, and formed high concentrations in sediment when water volume decreased, as previously reported. The upper course had the highest concentration of *T. latifolia* and *T. angustifolia*, followed by the middle and lower courses. The exposed littoral zone was flooded during the dry season, accompanied by emergent macrophytes that attracted animal grazing. As the draw-down progressed, it frequently accumulated droppings around the edges of the surrounding vegetation, with the severity increasing—organic matter accumulated due to the subsequent effects. Similar observations were made by Balarabe (2001) in Dumbi and Kwangila ponds, with increased *Typha* species at the pond's littoral borders, especially during the dry season. The increased organic carbon in the Kwangila above Dumbi was linked to intensive agricultural activity and fecal waste deposition. During the research period, all of these conditions could have contributed to the higher density of *T. latifolia* and *T. angustifolia* in the upper and middle courses. The water level variation was minor at the bottom course. Human activities were limited in type and scope with little or no cropland within the lower course and no vegetation except a few T. latifolia near the western littoral borders.

Impact of Typha *species on fish catch and distribution in Hadejia-Nguru Wetlands*

Fish in uninfested areas were more numerous and heavier than those caught in *Typha*-infested areas. This could be due to fish species migrating from *Typha*-infested to uninfested areas. Low dissolved oxygen (DO), high biological oxygen demand (BOD), high temperature, and transparency in a *Typha* species-infested area could all contribute to migration. This is consistent with Smith's findings from Delhi Lake, India, where *Typha australis* infestation resulted in the extinction or emigration of four families and twenty-two species of fish. Fish distribution may be influenced by changes in water level and temperature. Scheffer et al. (2003) found that reducing water volume raises fish harvest in Sudan Lake, which was corroborated by higher density found during the dry season. Similarly, favorable conditions in some regions of the wetlands may explain seasonal or yearly variations in composition and abundance. Daddy (2003) also found low catches in macrophytes-infested sections of the Tatabu flood plain of Niger state.

Management of Typha latifolia *and* T. angustifolia

Biological control with *P. karka* at various weights, manual control with cutting at various water levels, and physical control with shading at various tarpaulin numbers were all utilized to control Typha species throughout the two-year study period. Biological control

Biological control was used in the sampling site by utilizing varying weights of *P. karka*. After five months, the first observation was made. The number of tillers, leaves, and inflorescence decreased in each treatment, while nothing was observed in the control experiment. This is consistent with Smith (2004), who stated that the first effect of any control on the plant was observed in the leaves. It was also discovered that the more *P. karka* weight, the more successful the control. These effects could be related to the allopathic component found in the root of *P. karka*. Although the biological control is slow and gradual, it sometimes requires several years to manifest, its permanent effect is certain. Total biological control is the "only cost-efficient, permanent and environmentally friendly method." Greathead and Root (2010) confirmed that it takes several years for biological control agent monitoring to document their impact. However, significant successes have been reported using *Neochetina bruchi* in Argentina, Australia, India, and the USA (Harley 2000). In several countries, including the Sudanese systems of the Nile River, water hyacinth was controlled through biological control. In Nigeria, biological control with *Eichhornia bruchi* and *Neochetina bruchi* was utilized in the Kainji Dam, which reduced water hyacinth proliferation by 30% (Daddy 2006). The fascinating part about these discoveries is that *P. karka* could be used as animal fodder.

Manual control. Cutting at various levels for manual control: Cuttings were made at two levels below and above the water level. The results revealed that cuttings below the water level progressively died after 3-5 months, whereas *Typha* species cut above the water level gradually grew back. The lower the cutting depth below the water, the more effective it is, and the deeper the cutting depth, the higher the re-growth rate. This could be because lower cutting below water levels eradicates *Typha* species' rhizome. *Typha australis* stands are reduced when shoots of *Typha* species are clipped below the water surface in one growing season before flower formation (Weller 1975; Birnin-Yauri et al. 2004).

Physical control. Various quantities of the black tarpaulin were used. The results showed that the black tarp there were, the more successful the control was, and the fewer there were, the less effective the result was. This is due to *Typha* species being deprived of their ability to photosynthesize. It is also possible that the heat generated by the black tarpaulin is to blame. The best results were achieved in July to August, when *Typha* species' food supplies were thought to be the lowest. This is consistent with the findings of Nelson and Dietz (2006), who found that when actively growing *Typha* species tips were totally

covered for at least sixty days, they were killed. However, it was discovered that tremendous success was attained when the food supplies of *Typha* species were thought to be at their lowest, i.e., during the peak of the wet season, when nutrient dilution was at its highest.

To conclude, *T. latifolia* and *T. angustifolia* were discovered in the Hadejia-Nguru Wetlands, accounting for 65-70% and 30-35% of the total occurrence, respectively. The expansion of *Typha* species in the Hadejia Nguru wetlands was caused by water PO_4-p, NO_3-N, Magnesium, and sediment PO_4-p, NO_3-N, organic matter. The density, distribution, and consequently fish catches in Hadejia Nguru wetlands were reduced by *Typha latifolia* and *T. angustifolia*, as *Typha* uninfested area is more abundant by fish caught than the infested area in terms of the weight and number of fish caught. The optimum management approach for controlling *Typha* species in Hadejia Nguru wetlands, according to the study, is to cut the *Typha* below the water surface at a depth of 15cm.

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Effects of selected heavy metals on morphology of *Oreochromis niloticus* and *Clarias gariepinus* along Ruiru River, Kenya

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Abstract. *Ouma KO, Otieno SA, Sharma RR 2019. Effects of selected heavy metals on morphology of Oreochromis niloticus and Clarias gariepinus along Ruiru River, Kenya. Bonorowo Wetlands 9: 92-107.* The objective of this study was to determine the levels of heavy metals in tilapia and catfish species along the Ruiru River. Sediments, water, and fish samples were collected using systematic random sampling techniques. Three sites were established downstream of the river, 1000 meters from Ruiru Town. The other three were established upstream of the river, 1000 meters from Ruiru Town. Fish samples were transported to the National Museums of Kenya laboratory for identification. An atomic absorption spectrophotometer was used to examine metals. Fish gills and livers were also examined for histopathological changes. Using one-way analysis of variance, researchers analyzed data on heavy metal levels in the water, sediments, fish gills, livers, and muscles. Correlation coefficients were also calculated to assess the relationship between fish length and weight and metal concentrations in fish liver, gills, and muscles and between levels of heavy metals in water and sediments. In April, 0.167±0.014 mg/L, August, 0.054±0.003 mg/L, and December, 0.222±0.101 mg/L ($F = 2.10$, $p = 0.202$), mean chromium levels in water were not statistically significant, but were statistically significant at the downstream sites 0.236±0.019 mg/L, 0.058±0.001 mg/L, and 0.222±0.101 mg/L during the three months ($F = 125.63$, $p<0.001$). Significant positive correlations between the levels of iron in sediments and water were found at both upstream and downstream sites, with $r = 0.7319$, $p = 0.025$, and $r = 0.8506$, $p = 0.0037$, respectively. The linkage between lead levels in sediments and water at upstream sites was not significant ($r = 0.343$, $p = 0.366$), while it was significant ($r = 0.7523$, $p = 0.019$) at downstream sites. Chromium levels in sediments and water at upstream sites indicated a positive but non-significant connection ($r=0.5339$, $p=0.138$). There was a substantial positive correlation between chromium levels in sediments and water ($r = 0.9787$, $p<0.001$). Metals accumulated in fish tissues in the following order: liver>gills>muscle, and *Clarias gariepinus* tissues from downstream sites exhibited higher amounts of metals than *Oreochromis niloticus* tissues from the same sites. Both kinds of fish exhibited higher iron levels than lead in all tissues, with chromium being the lowest. The liver and gills of fish from the upstream research sites had normal features on histology. Hepatocytes with larger nuclei were found in fish livers from downstream sites. The secondary lamellae of the gills had degenerated and fused. According to the study, metal levels in sediments were greater than in water. Fish from the downstream sites also showed morphological alterations in the liver and gills. The study's findings suggest that people who eat such fish may be exposed to metal poisoning. According to the report, the National Environment Management Authority should implement measures to reduce industrial trash flow into the Ruiru River.

Keywords: *Clarias gariepinus*, heavy metals, morphology, *Oreochromis niloticus*, Ruiru River

INTRODUCTION

Ruiru Town is located on the banks of the Ruiru River, which originates on the slopes of the Aberdare Ranges. Along the channels and in the catchments, it is exposed to pollutants and experiences various anthropogenic consequences (Bundambula and Mwachiro 2006). Intensive agriculture, animal husbandry, silviculture, horticulture, charcoal burning, and quarrying are the principal human activities in the catchment (UNEP 2001). Large amounts of contaminants are carried downstream by water drainage from upstream districts (Mwenda and Guthiga 2010). Agricultural runoff containing organic wastes, fertilizers, pesticides, and weed killers is thrown into the river, posing a concern (UNEP 2001). The river continues to be polluted as it passes through the highly populated Ruiru Municipality, which lacks sufficient sewage infrastructure, a wastewater treatment facility, and solid waste disposal systems (UNEP 2001). Residential areas, industrial zones, the marketplace, and the commercial central business district are the primary wastewater sources in Ruiru Town (CBD). In addition to agricultural operations, Ruiru town has several factories, including Devki Steel Mills, Spinners and Spinners Garment Factory, and Ruiru Feeds (EIA Report 2011). Most of the town's wastewater is dumped into the Ruiru River with little or no treatment. In aquatic ecosystems, the presence of heavy metal pollutants over natural loads has become a growing concern (UNEP 2001). The rapid growth of populations, industrial development and the discharge of untreated industrial wastes, growing urbanization, expansion of natural resources, irrigation expansion, and contemporary agricultural methods have all contributed to this predicament (UNEP 2001). Concerns regarding the health of aquatic species as a result of pollution have grown in recent years. Pollution of rivers has been caused by industrial effluents, raw sewage, and trash from human settlements along rivers, posing a health risk to those communities and putting a strain on the aquatic ecosystem (UNEP 2001).

The global food crisis has impacted Kenya and other African countries. According to a Food and Agriculture Organization report, high food costs are a global issue (Ministry of Agriculture 2009). The urban poor and small-scale farmers have been identified as two of the most vulnerable populations. Fish like tilapia, catfish and common carp have been reported in the Ruiru River (UNEP 2001). Anecdotal evidence suggests that fishing occurs on occasion along the river. Fish in the aquatic food chain bioaccumulate and store organic and inorganic contaminants in organs like the liver, gills, and kidneys, which can then be transferred down the food chain to other organisms consuming fish, such as birds. Fish play a crucial role in maintaining the biological balance of plankton colonies and other aquatic invertebrates. They can concentrate huge amounts of metals from the water (Abdulali et al., 2011).

The aims of this study were (i) To determine the concentration of lead, iron, and chromium in water and sediments in Ruiru River, Kenya (ii) To determine lead, iron, and chromium concentrations in the gills, liver, and muscles of *Clarias gariepinus* and *Oreochromis niloticus* in the Ruiru River, (iii) To assess the morphological effects of lead, iron, and chromium on the gills and liver of tilapia and catfish in Ruiru River.

MATERIALS AND METHODS

Study area

The research was conducted near the Ruiru River in Kiambu County, Kenya. During the investigation, six major sampling locations along the river were sampled. The river rises on the Aberdare Range's foothills and passes past various coffee farms before reaching Ruiru Town. Ruiru Town is situated in a transitional zone on the border between the Upper Athi Basin and the Kikuyu Plateau. The topography is undulating in general, with a drainage system that leads to the Athi river basin. The township is split in two by the Ruiru River. The town's geography is mainly hilly to the north-west, and the Mukuyu and Ruiru Rivers cut through it (EIA Report 2011). These areas are characterized by housing and industrial growth in addition to agriculture activities (EIA Report 2011). Despite the enormous population, many informal settlements along the river with no adequate sewage or solid waste disposal infrastructure. The river also runs through locations where certain industries pour their waste (UNEP 2001). As a result, human activities along the Ruiru River impact wildlife living in the river's downstream zones. Longitudes 36055'52” and 3701'18” and latitudes 107'54” and 109'50” were used to sample the area (Figure 1). The climate can be classified as tropical. The climate and temperatures within the research region are influenced by height because these sites are located on the slopes of the Aberdare Range, with colder zones to the north and drier zones to the south. The average annual rainfall ranges from 600mm to 1100mm. Temperatures are generally hot, with an average yearly temperature of 18°C to 20°C. The landscape in the research region is very gentle, with a general descent towards the Athi River.

However, the higher hillnorthwestorth west has a deeply dissected topography with numerous streams and ridges, while the lowlands to the southeast have fewer streams, shallower, and broader valleys. The average elevation is around 1520 meters above sea level. Agriculture is an important source of income in Ruiru. The county government's agricultural activities include crop and animal husbandry, livestock sale yards, county abattoirs, plant and animal disease management, and fisheries (Kiambu County Government Report 2014). Climate conditions are favorable for important cash crops such as coffee and horticulture (Makokha et al. 2001). Crops are farmed for both personal and commercial interests. The main cash crop cultivated in this area is coffee, which is processed locally, and the excess is exported. Horticulture and floriculture farming, which is mainly done in greenhouses, are also conducted by some farmers. Red Lands Roses, for example, based in Ruiru since 1996, specializes in cultivating and exporting T-Hybrid and spray roses in over 100 kinds in greenhouses. Cereals like maize and beans are mainly used for household consumption, but surpluses are frequently shipped to surrounding areas like Nairobi and Thika. Dairy cattle are raised to produce milk and other dairy products. Following the government's Economic Stimulus Program (ESP), fish aquaculture has developed in this area (The Star 2011). Ruiru is well-covered by industries that have supplied labor and contributed to the town's economic development. There are currently various flower businesses and coffee factories in operation, which both commercial enterprises and cooperative societies hold. The majority of these factories are located around the Ruiru River. Textiles, plastics, chemicals, food processing, and steel industries are the key industries of Ruiru. Study design

The research design used in this study was comparative. Gill nets were used to catch the fish, and plastic bottles collected water samples. Lead, iron, and chromium levels in water and sediment samples obtained from sampling locations along the Ruiru River's downstream were compared to levels in samples collected from three study sites upstream of Ruiru Town, which served as a control. The amounts of lead, iron, and chromium in the gills, liver, and muscles of *O. niloticus* and *C. gariepinus* were determined. Fish tissues and organs were compared to those from reference sites on the upstream parts of the river that were not exposed to high amounts of pollution. The morphology of the gills and liver of fish sampled from the downstream components of the river was evaluated, and a comparison was performed with fish sampled from the reference sites.

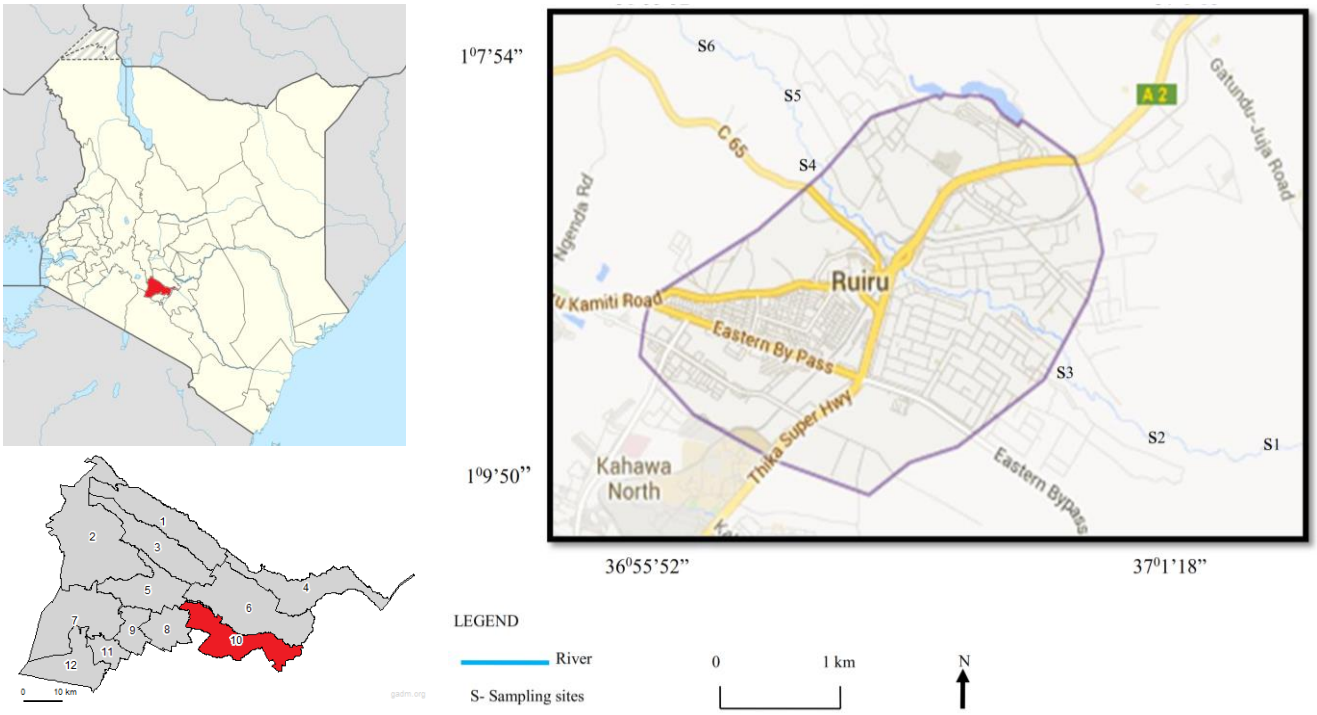

Figure 1. Map showing sampling sites along the course of Ruiru River in Kiambu County, Kenya

Sampling and sample size

Concerning Ruiru Town, the course of the Ruiru River was partitioned into downstream and upstream areas, with three sampling sites chosen in each area, for a total of six sampling sites along the river, S1, S2, S3, S4, S5, and S6 (Figure 1). The first, second, and third sampling sites, designated as S1, S2, and S3, were positioned 1000 meters downstream of Ruiru Town. In contrast, the three control sites, designated as S4, S5, and S6, were located 1000 meters upstream of Ruiru Town. The sampling points were separated by 100 meters. Locations of effluent discharge into the river, ecosystem types, points of confluence between the Ruiru River and other rivers, and sample site proximity to settlements were all considered while selecting sampling sites. The physical appearances of the river water, land-use patterns, economic activity, and the physical look of the river water were all considered. Each sampling location measured 100 meters in length.

The distance between sampling sites was set at 100 meters before the survey began, based on the levels of pollution, the geology of the area, and the processes that influence the current sediment distribution, such as erosion, transportation, and accumulation. During the preliminary studies, sites with sufficient samples were purposefully identified. During each month of the study, six water samples, six composite sediment samples, and eighteen samples of each fish species were taken from the river.

During December 2012, April 2013, and August 2013, samples were collected once a month. The three months were chosen to account for seasonal changes in iron, lead, chromium levels, sediments, and fish tissues investigated in water, sediments, and fish tissues.

Water quality parameters

During each sampling water and sediments session, water quality parameters were determined at each sampling site. Hanna's digital meter was used to measure temperature, pH, and electrical conductivity.

Collection of water samples

Each sample bottle was dipped 30 centimeters below the water surface, and the open end of the container was projected against the flow direction during sampling. Water samples from each site were collected in clean 250-milliliter plastic bottles for heavy metals analysis.

Concentrated nitric acid was applied to lower the pH to 2 and preserve the water samples until they were examined for iron, lead, and chromium at Kenyatta University's Department of Chemistry. Collection of sediment samples

The Riverbank sampled sediments at the water sampling locations. These were taken directly from the surface (5–20 cm) with a hand-held trowel. At each sampling site, two equal amounts of sediment were obtained and homogenized thoroughly in a plastic container to create a combination representative of the area tested, yielding a total of six composite samples from the downstream and upstream research sites throughout the Ruiru River's course. Surface water was decanted from the sample during the operation while keeping the fine sediment component. The composite sediment samples were then transferred to designated plastic containers using a plastic laboratory spoon.

Sampling of fish

Fish samples were collected using gill nets. The nets were 20 feet long and 5 feet high, with 3-inch mesh, and were made with floats on the top horizontal line and weights on the bottom horizontal line. The nets were dropped down at the sampling sites at 9.00 a.m. and picked up at 3.00 p.m. Fish samples were sent to Kenya's National Museums for identification. *O. niloticus* and *C. gariepinus* were the targeted fish. During each month of sampling, three fish of each species were collected from each of the six sampling sites, for a total of eighteen samples of each fish species. The total length of each fish was measured in centimeters, and its wet body weight was determined in grams using a digital electronic balance immediately after collection. The studied fish ranged from 15 to 30 centimeters in length and weighed between 30 and 180 grams.

Preparation and analysis of heavy metals in water samples

To ensure that metals do not attach to the container's walls, a preservative, nitric (V) acid (HNO_3), was added to the original sample. After vigorous shaking to guarantee suspension of any materials that may have settled, sample aliquots for digestion were obtained. On a heated plate, water samples were digested with hydrochloric acid (HCl) and HNO_3 in a volume: volume ratio (1%: 0.5%). At Kenyatta University's Department of Chemistry, digested samples were filtered, diluted to 100 cm3, and then evaluated for lead, iron, and chromium using a Buck Scientific Atomic Absorption Spectrophotometer model 210 VGP. Each sampling station provided three water samples, which were evaluated. Preparation and analysis of heavy metals in sediment samples

In the laboratory, samples were air-dried at room temperature. After air-dried, sediment samples were pulverized and sieved using a 160 m sieve. The sediment samples were then weighed and placed in digestion flasks with 10 milliliters of HNO_3/HCl (1:3 v/v) and digested in the fume chamber on a hot plate. At Kenyatta University's Department of Chemistry, samples were evaluated for lead, iron, and chromium using a Buck Scientific Atomic Absorption Spectrophotometer model 210 VGP.

Preparation and analysis of heavy metals in fish tissues

Muscle, gills, and liver were removed from the fish and dried separately in an oven at 80°C for two days to achieve a constant dry weight. These were pulverized with a porcelain pestle and mortar (Poldoski, 1980). Half a gram of powdered muscle and gill samples, as well as 0.1 gram of dry weight liver samples, were digested with 3 milliliters of HNO3 (65%) and 1-milliliter hydrogen peroxide (35 %). The samples were then transferred to volumetric flasks and diluted with deionized water to 50 milliliters for muscle and gills and 25 milliliters for liver samples due to the smaller quantity of liver samples, then filtered with Whatman filter paper. Using an Atomic Absorption Spectrophotometer, the concentrations of lead, iron, and chromium were determined. The amounts of these metals in water, sediment, and fish tissues were compared in the downstream and upstream sections of the Ruiru River and the maximum allowable levels set by the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA).

Histological procedures

Fish were dissected, and sections of gills and liver were removed and fixed for 48 hours in Bouin's solution. The tissues were dehydrated in four steps using a graded series of ethanol solutions in glass vials: 50 %, 70 %, 90 %, and two changes of 100 % ethanol. Xylene was used to remove ethanol from the tissues. Tissues were then paraffin-infiltrated, embedded in paraffin wax blocks, and sectioned at 5 microns using a microtome. To remove creases, cut sections were floated in a water bath kept at 56°C, then picked up on glass microscope slides. Paraffin wax was removed from the tissue by running the slides through xylene before staining. Tissues were then hydrated in increasing ethanol concentrations, stained with hematoxylin and eosin stains, and viewed under a light microscope at magnifications of 150 and 400.

Control

Comparison of levels of iron and lead in water and sediments collected from the downstream sections of the Ruiru River with levels in samples obtained from the control sections of the river identified as S4, S5, and S6 (Figure 1) based on the assumption that they were not contaminated with lead, iron, and chromium arising from industrial wastes and human activities in Ruiru Town. Fish liver and gill histology sections from downstream river sections were compared to those from the control sites.

Data analysis

One-way analysis of variance was used to examine lead, iron, and chromium levels in water, sediments, fish gills, liver, and muscles. The correlation coefficient was also performed to analyze the relationship between biotic parameters such as fish weight and length and metal concentrations in the gills, liver, and muscles of the fish. It was also utilized to establish a link between heavy metal concentrations in water and sediments and fish gills, liver, and muscles.

RESULTS AND DISCUSSION

Water quality parameters

The lowest temperature values along the Ruiru River were reported in April at upstream study sites (20.33 ± 0.14°C) and downstream study sites (21.22 ± 0.17°C) (Table 1). There was no statistically significant variation in water temperature between the upstream and downstream study locations along the river during this month, $p = 0.001$. Temperature values were found to be substantially lower at upstream study sites (20.89 ± 0.18°C) than downstream study sites (21.67 ± 0.12°C) throughout August, $p = 0.0025$. The highest temperatures were reported at the upstream (23.33 ± 0.12°C) and downstream (24.22 ± 0.17°C) study locations during December (Table 1). During December, the temperature differences between

the two research sites were likewise statistically significant ($p = 0.0005$). At both the upstream ($F = 112.54$, $p < 0.001$) and downstream ($F = 110.78$, $p < 0.001$) study sites, the mean differences in temperature values throughout the three months were found to be statistically significant (Table 1).

The pH of the water in the Ruiru River was found to be mildly acidic, with the lowest mean values recorded in April at the upstream (5.97 ± 0.02) and downstream (5.87 ± 0.02) research sites. The pH difference between the two study sites was statistically significant ($p = 0.0026$). The greatest values were found at the upstream (6.79 ± 0.01) and downstream (6.53 ± 0.02) research sites along the river during August. In August, the changes in pH mean values were likewise considerably different ($p < 0.001$). In December, the pH value at the downstream study site (6.10 ± 0.07) was found to be substantially lower than at the upstream study site along the river (6.51 ± 0.07), $p < 0.001$). Seasonal fluctuations in pH mean values were statistically significant at both the upstream ($F = 99.58$, $p < 0.001$) and downstream ($F = 59.14$, $p < 0.001$) study sites along the river during the three months (Table 1).

The lowest mean electrical conductivity (EC) values in Ruiru River were obtained in April at upstream study sites (424.00 ± 0.88 S/cm) and downstream research sites (592.11 ± 1.72 S/cm). Differences in electrical conductivity mean values were statistically significant (p 0.001). The mean electrical conductivity at upstream study sites along Ruiru River was 761.11 ± 8.80 S/cm in August, while the value at downstream study sites was 1003.56 ± 11.57 S/cm. The mean electrical conductivity differences between the two sites were found to be statistically significant ($p < 0.001$) (Table 1). During December, the downstream sites had the highest electrical conductivity values. The mean values of electrical conductivity recorded at the upstream (839.78 ± 10.91 S/cm) and downstream study locations (1162.00 ± 10.31 S/cm) were significantly different in December ($p < 0.001$). There were also significant differences in mean electrical conductivity values at upstream study sites ($F = 741.83$, $p < 0.001$) and downstream study sites ($F = 1067.07$, $p < 0.001$) along the river throughout April, August, and December (Table 1).

The mean total dissolved solid (TDS) value recorded at upstream study sites in April (34.67 ± 0.94 mg/L) was statistically lower than the values recorded at downstream study sites (72.11 ± 1.48 mg/L) ($p < 0.001$). TDS mean values at the upstream (38.89 ± 1.21mg/L) and downstream (56.11 ± 1.27mg/L) research locations were likewise statistically significant ($p < 0.001$) in August. The mean TDS values at the downstream study sites (68.00 ± 1.41 mg/L) were substantially higher in December than at the upstream study sites along Ruiru River (54.89 ± 0.75 mg/L), $p < 0.001$. Seasonal fluctuations in mean TDS values in Ruiru River throughout the three months differed considerably both upstream ($F = 117.16$, $p < 0.001$) and downstream ($F = 35.69$, $p < 0.001$) study sites (Table 1).

Levels of iron in sediments along Ruiru River

Between April and December, there was an overall increase in iron levels in sediments at both upstream and downstream study sites along the river. In April, August, and December, the differences in mean iron values in sediments at the upstream and downstream research locations were not statistically significant. However, across the three months, the amounts of iron in sediments were considerably higher in December, followed by August and then April, both upstream ($F = 399.84$, $p < 0.001$) and downstream ($F = 574.95$, $p < 0.001$) along Ruiru River (Figure 2).

Levels of iron in the water

The mean amounts of iron in water samples collected from downstream sampling sites were greater in April and August than in samples collected from upstream sampling sites, albeit the differences were not statistically significant. The mean levels of iron in water samples from downstream research sites along Ruiru River (4.26 ± 0.21mg/L) were substantially higher in December than the mean levels of iron observed at upstream study sites along the river (2.81 ± 0.21mg/L), ($p = 0.007$). These were the highest iron levels found in water samples taken along the river. At the upstream study sites ($F = 10.17$, $p = 0.012$), and also at the downstream study sites along the river ($F = 141.90$, $p < 0.001$), levels of iron in water were substantially higher in December, followed by April, then August (Figure 3).

Table 1. Mean values (±SD) for water quality parameters at the upstream and downstream sampling sites along Ruiru River during April, August, and December.

		Temp (°C)	pH	Electrical conductivity (µS/cm)	TDS (mg/L)
April	Upstream	20.33±0.14	5.97±0.02	424.00±0.88	34.67±0.94
	Downstream	21.22±0.17	5.87±0.02	592.11±1.72	72.11±1.48
	P value	0.001	0.0026	<0.001	<0.001
August	Upstream	20.89±0.18	6.79±0.01	761.11±8.80	38.89±1.21
	Downstream	21.67±0.12	6.53±0.02	1003.56±11.57	56.11±1.27
	P value	0.0025	<0.001	<0.001	<0.001
December	Upstream	23.33±0.12	6.51±0.07	839.78±10.91	54.89±0.75
	Downstream	24.22±0.17	6.10±0.07	1162.00±10.31	68.00±1.41
	P value	0.0005	<0.0008	<0.001	<0.001

Note: TDS: Total dissolved solids

Levels of lead in sediments

Between April, August, and December, the lead levels in sediments at both upstream and downstream study locations along the Ruiru River increased. In April, August, and December, the differences in mean lead levels in sediments at the upstream and downstream research sites were not statistically significant. However, lead levels in sediments were found to be considerably higher in December, followed by August, and then April at both upstream ($F = 43.10$, $p < 0.001$) and downstream ($F = 41.84$; $p < 0.001$) study sites along Ruiru River (Figure 4).

Levels of lead in water

In April and August, the mean lead levels in water variations between the upstream and downstream research locations were not statistically significant. The mean levels of lead in water at downstream sampling sites along Ruiru River (4.07 ± 0.53mg/L) were considerably higher in December than the mean levels of lead in water at upstream sampling sites (1.88 ± 0.54mg/L), ($p = 0.044$) (Figure 5). According to this study, the changes in lead levels in water over the three months of April, August, and December were not statistically significant at the upstream study locations along Ruiru River ($F = 1.501$, $p = 0.296$). However, lead levels in the water were considerably higher in December at the downstream study sites, followed by April and August ($F = 33.199$, $p = 0.0006$) (Figure 5).

Levels of chromium in sediments

Chromium levels were lower in sediments from upstream sections along the Ruiru River than in samples from downstream sections. In April, chromium levels in sediments at downstream study sites along the river (3.27 ± 0.25mg/kg) were substantially higher than chromium levels in sediments at upstream study sites along the river (1.86 ± 0.24mg/kg), ($p = 0.015$) (Figure 6). In August and December, the differences in mean chromium levels in sediments at the upstream and downstream study sites were not statistically significant. However, when chromium levels in sediments were compared by month of sampling, it was found that levels were considerably higher in December, followed by April, and then August at upstream study sites ($F = 14.48$, $p = 0.0051$), as well as downstream study sites ($F = 64.20$, $p = 0.0001$) (Figure 6).

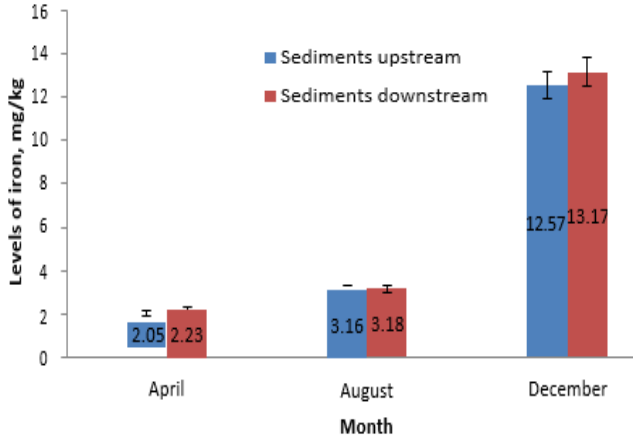

Figure 2. Levels of iron in sediments (mg/kg) during April, August, and December at the upstream and downstream study sites along Ruiru River.

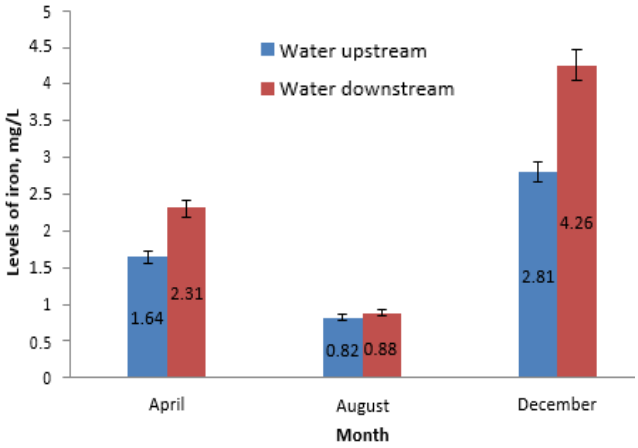

Figure 3. Levels of iron in the water (mg/L) during April, August, and December at the upstream and downstream study sites along Ruiru River.

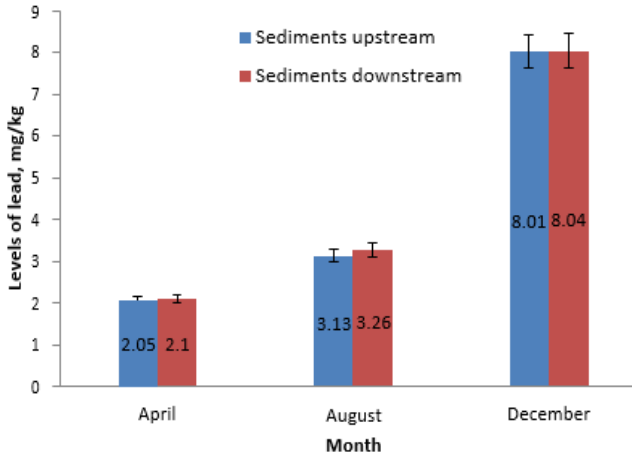

Figure 4. Lead levels in sediments (mg/kg) during April, August, and December at the upstream and downstream study sites along Ruiru River.

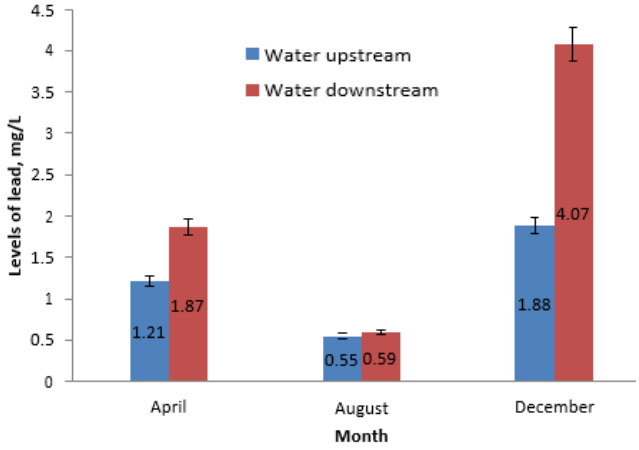

Figure 5. Levels of lead in water (mg/L) during April, August, and December at the upstream and downstream study sites along the Ruiru River

Levels of chromium in water

The downstream study sites along the Ruiru River had greater chromium levels than the upstream sampling sites. In April, the levels of chromium in water at the downstream study sites (2.36 ± 0.19 mg/L) were substantially higher than the levels of chromium in water at the upstream research sites (1.67 ± 0.14 mg/L) ($p = 0.045$) (Figure 7). In December, researchers discovered that the differences in mean chromium levels in water at the upstream study site (2.22 ± 1.01 mg/L) and downstream study sites (5.30 ± 0.31 mg/L) were statistically significant ($p = 0.043$). At the downstream study sites, chromium levels in the water were considerably higher in December, followed by April, and then August ($F = 125.63$, p 0.001) (Figure 7).

Correlation between levels of iron, lead, and chromium in sediments and water during the three months of sampling

During April, August, and December, there was a significant positive correlation between iron levels in sediments and water at the upstream study sites along the Ruiru River ($r = 0.7319$, $p = 0.025$) (Table 2).

There was also a significant positive correlation ($r = 0.8506$, $p = 0.0037$) in the downstream sites along the Ruiru River, indicating that high iron levels in the sediments grew as metal levels in water increased (Table 2). At the upstream study sites, there was no significant correlation between lead levels in sediments and water ($r = 0.343$, $p = 0.366$) (Table 2). However, there was a significant correlation ($r = 0.7523$, $p = 0.019$) at the downstream study sites (Table 2).

The levels of chromium in sediments and water along the Ruiru River exhibited a positive correlation ($r = 0.5339$, $p = 0.138$); however, it was not statistically significant (Table 2). The value of r was 0.9787 in the downstream study sites along the river. This is a substantial positive correlation, indicating that greater chromium levels in sediments increased as chromium levels in water increased ($p < 0.001$) (Table 2).

Levels of metals in the tissues of *C. gariepinus* and *O. niloticus were* sampled from the upstream and downstream study sites along Ruiru River.

Although the differences were not significant, upstream research sites along the Ruiru River, *C. gariepinus*, had greater mean levels of the three metals than *O. niloticus* (Table 3).

The findings also demonstrate that all *C. gariepinus* tissues collected from downstream research sites along the Ruiru River had higher amounts of all the examined metals than *O. niloticus* tissues collected from the same sites. Both investigated fish species exhibited higher iron levels than lead in all tissues. Chromium has the lowest concentration of all the metals tested. Furthermore, both fish species collected heavy metals in their tissues in a similar pattern. The liver carries higher levels of metals than the gills and muscles containing the lowest levels (Table 3).

Table 2. Relationship between levels of metals in sediments (mg/kg ± SD) and in water (mg/L ± SD) at the upstream and downstream study sites along Ruiru River during April, August, and December.

	Sediments	Water	r	p
Upstream				
Iron	5.81±1.62	1.75±0.32	0.731	0.025
Lead	4.39±0.94	1.21±0.33	0.3429	0.366
Chromium	2.52±0.60	1.47±0.38	0.534	0.138
Downstream				
Iron	6.19±1.75	2.48±0.49	0.8506	0.0037
Lead	4.46±0.94	2.17±0.53	0.7523	0.01935
Chromium	3.58±0.68	2.74±0.69	0.9787	0.000

Note: Correlation value is significant at $P \leq 0.05$, Pearson linear correlation, $\alpha = 0.05$

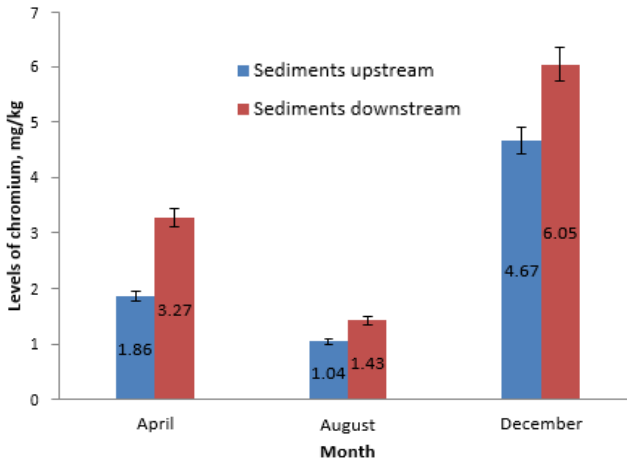

Figure 6. Levels of chromium in sediments (mg/kg) during April, August, and December at the upstream and downstream sampling sites along the Ruiru River

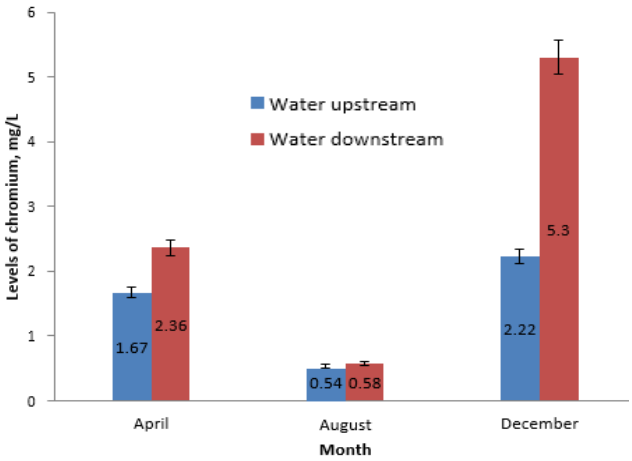

Figure 7. Levels of chromium in water (mg/L) during April, August, and December at the upstream and downstream study sites along Ruiru River.

Table 3. Mean levels of iron, lead, and chromium (mg/kg ± SD) in the tissues of *Clarias gariepinus* and *Oreochromis niloticus* sampled from the upstream and downstream study sites along Ruiru River.

Part of the body	Fish species	Fe (mean±SE) mg/kg	Pb (mean±SE) mg/kg	Cr (mean±SE) mg/kg
Upstream				
Liver	*C. gariepinus*	3.67±0.54	0.84±0.30	0.62±0.18
	O. niloticus	2.71±0.44	0.555±0.21	0.50±0.14
p-value		0.1792	0.4834	0.619
Gills	*C. gariepinus*	2.73±0.54	0.26±0.12	0.27±0.1
	O. niloticus	1.85±0.30	0.26±0.10	0.15±0.04
p-value		0.165	0.9974	0.295
Muscles	*C. gariepinus*	2.11±0.46	0.25±0.07	0.16±0.06
	O. niloticus	1.38±0.23	0.15±0.05	0.13±0.04
p-value		0.166	0.278	0.679
Downstream				
Liver	*C. gariepinus*	5.24±0.86	1.36±0.46	0.68±0.19
	O. niloticus	3.55±0.58	0.69±0.28	0.56±0.15
p-value		0.112	0.225	0.631
Gills	*C. gariepinus*	3.17±0.50	0.75±0.26	0.33±0.11
	O. niloticus	2.08±0.30	0.44±0.14	0.17±0.06
p-value		0.072	0.306	0.211
Muscles	*C. gariepinus*	2.34±0.42	0.46±0.15	0.20±0.06
	O. niloticus	1.68±0.25	0.29±0.09	0.15±0.04
p-value		0.183	0.349	0.5619

Table 4. Mean levels of iron, lead, and chromium (mg/kg ± SD) in tissues of *Clarias gariepinus* and *Oreochromis niloticus* from upstream and downstream study sites along Ruiru River during April, August, and December.

		Liver	Gills	Muscles
Clarias gariepinus				
Iron	Upstream	3.67±0.55	2.73±0.54	2.11±0.46
	Downstream	5.24±0.87	3.17±0.50	2.34±0.42
	p	0.132	0.277	0.718
Lead	Upstream	0.84±0.3	0.26±0.12	0.25±0.07
	Downstream	1.36±0.4	0.75±0.26	0.46±0.15
	p	0.352	0.099	0.227
Chromium	Upstream	0.62±0.18	0.27±0.1	0.16±0.06
	Downstream	0.68±0.19	0.33±0.11	0.20±0.06
	p	0.812	0.670	0.697
Oreochromis niloticus				
Iron	Upstream	2.71±0.44	1.85±0.30	1.38±0.23
	Downstream	3.55±0.58	2.09±0.30	1.68±0.25
	p	0.254	0.592	0.398
Lead	Upstream	0.55±0.21	0.26±0.10	0.15±0.05
	Downstream	0.69±0.28	0.44±0.14	0.29±0.09
	p	0.694	0.329	0.202
Chromium	Upstream	0.50±0.14	0.15±0.04	0.13±0.04
	Downstream	0.56±0.15	0.17±0.06	0.15±0.04
	p	0.771	0.823	0.761

Table 5. Relationship between iron levels (mg/kg) in selected tissues and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites.

	Upstream		Downstream	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Clarias gariepinus				
Liver	r = 0.151	r = 0.171	r = 0.153	r = 0.147
	p = 0.452	p = 0.393	p = 0.466	p = 0.465
\Gills	r = 0.249	r = 0.354	r = 0.364	r = 0.269
	p = 0.210	p = 0.070	p = 0.062	p = 0.174
Muscles	r = 0.266	r = 0.361	r = 0.317	r = 0.194
	p = 0.180	p = 0.064	p = 0.107	p = 0.332
Oreochromis niloticus				
Liver	r = 0.535	r = 0.358	r = 0.510	r = 0.618
	p = 0.004**	p = 0.067	p = 0.007**	p = 0.001 **
Gills	r = 0.504	r = 0.343	r = 0.519	r = 0.633
	p = 0.007**	p = 0.080	p = 0.006**	p = 0.001**
Muscles	r = 0.452	r =0.296	r = 0.438	r = 0.507
	p = 0.018**	p = 0.134	p = 0.022*	p = 0.007**

Note: * Correlation value is significant at $p \leq 0.05$, Pearson linear correlation, $\alpha = 0.05$

Levels of iron, lead, and chromium in tissues of *Clarias gariepinus* and *Oreochromis niloticus* from the upstream and downstream sites along Ruiru River.

All three heavy metals were found in higher concentrations in the tissues of *C. gariepinus* fish obtained from downstream study sites than in fish collected from upstream study sites along the river. However, the variations in the mean were not statistically significant (Table 4). The mean levels of iron, lead, and chromium in the liver, gills, and muscles of *O. niloticus* taken from upstream and downstream research sites along the Ruiru River made no significant change (Table 4).

Relationship between levels of iron and fish morphometrics in *Clarias gariepinus* And *Oreochromis niloticus*

In *C. gariepinus* sampled from both upstream and downstream study sites along the Ruiru River, the correlation between iron levels in the fish liver, gills, and muscles and fish length and weight indicated favorable relationships. The correlations, however, were not statistically significant (Table 5).

In *O. niloticus* sampled from both upstream and downstream study sites along the Ruiru River, the linkage between iron levels in the fish liver, gills, and muscles and fish length and weight indicated positive relationships. There was a significant positive correlation between the levels of iron in fish liver and the length of fish in *O. niloticus* sampled from upstream study locations ($r = 0.535$, $p = 0.004$). Although there was a positive correlation between fish liver iron levels and fish weight from the same research site ($r = 0.358$, $p = 0.067$), the correlation was not statistically significant. There were substantial

positive correlations between the levels of iron in fish liver and both length (r = 0.510, p = 0.007) and weight (r = 0.618, p = 0.001) of fish taken from the downstream research sites (Table 5).

The levels of iron in the gills of *O. niloticus* captured from upstream research locations were significantly correlated to length (r = 0.504, p = 0.007), but not to weight (r = 0.343, p = 0.080). However, there were substantial positive relationships between the levels of iron in *O. niloticus* gills and both length (r = 0.519, p = 0.006) and weight (r = 0.633, p = 0.001) in fish obtained from downstream research locations along the Ruiru River (Table 5).

The findings also show a substantial positive correlation between iron levels in *O. niloticus* muscles and fish length (r = 0.452, p = 0.018) in fish acquired from upstream study sites, whereas a positive but not significant correlation between levels of iron in the muscles and fish weight. In fish gathered from downstream research locations, significant positive correlations were found between levels of iron in the muscles of *O. niloticus* and length (r = 0.438, p = 0.022) and weight (r = 0.507, p = 0.007) (Table 5).

Relationship between levels of lead and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites

The lead levels in the liver, gills, and muscles of *C. gariepinus* were positively associated with both length and weight in fish sampled from upstream study sites and length and weight in fish taken from downstream research sites in this investigation. However, the connections were not statistically significant (Table 6). Relationship between levels of lead and fish morphometrics in *O. niloticus* at the upstream and downstream study sites

The lead levels in fish liver, gills, and muscles were positively correlated to fish length, and weight in *O. niloticus* sampled from upstream study sites along Ruiru River; however, the correlations were not statistically significant (Table 6).

Relationship between levels of chromium and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites

Similarly, levels of chromium in the tissues of *C. gariepinus* and *O. niloticus* in fish samples from upstream and downstream study sites were positively correlated with both fish length and weight. The correlations, on the other hand, were not statistically significant. Morphological effects of iron, lead, and chromium in the liver and gills of *C. gariepinus* and *Oreochromis niloticus.*

The levels of iron, lead, and chromium in water and sediments in the Ruiru River were connected to morphological alterations in the gills and liver of *C. gariepinus* and *O. niloticus* in the current study. Fish liver histology at the upstream research site revealed normal liver structure with no pathological abnormalities. A continuous mass of huge hexagonal hepatic cells makes up the liver of a fish. Hepatic cells were polygonal, with spherical nuclei either exocentric or slightly centrally located (Figures 8 and 9).

Table 6. Relationship between levels of lead (mg/kg) in selected tissues and fish morphometrics in *Clarias gariepinus* and *Oreochromis niloticus* at the upstream and downstream study sites.

	Upstream		**Downstream**	
	Length (cm)	**Weight (g)**	**Length (cm)**	**Weight (g)**
Clarias gariepinus				
Liver	r = 0.241	r = 0.156	r = 0.068	r = 0.021
	p = 0.225	p = 0.437	p = 0.708	p = 0.916
Gills	r = 0.157	r = 0.191	r = 0.097	r = 0.129
	p = 0.435	p = 0.340	p = 0.631	p = 0.522
Muscles	r = 0.264	r = 0.306	r = 0.211	r = 0.182
	p = 0.184	p = 0.121	p = 0.291	p = 0.365
Oreochromis niloticus				
Liver	r = 0.209	r = 0.008	r = 0.288	r = 0.31
	p = 0.295	p = 0.970	p = 0.145	p = 0.115
Gills	r =0.16	r = 0.153	r = 0.137	r = 0.237
	p = 0.408	p = 0.445	p = 0.495	p = 0.235
Muscles	r = 0.248	r = 0.116	r = 0.317	r = 0.288
	p = 0.212	p = 0.563	p = 0.107	p = 0.146

Note: * Correlation value is significant at $p \leq 0.05$, Pearson linear correlation, $\alpha = 0.05$

Hepatocytes with loss of normal arrangement were found in the livers of *C. gariepinus* and *O. niloticus* from the downstream regions of the Ruiru River. Fish livers showed larger hepatocytes with expanded nuclei (Figures 10 and 11).

Clarias gariepinus and *O. niloticus* gills collected from upstream study sites along the Ruiru River have normal gill structures. Each arch featured two rows of primary gill filaments connected at the base by a gill septum. Primary gill lamellae featured central supporting axes and rows of secondary gill lamellae on both sides (Figures 12 and 13). Fish gills from downstream areas of the river, on the other hand, showed proliferation of interlamellar epithelial cells, as well as hyperplasia and fusion of the secondary lamellae (Figures 14 and 15).

Permissible values of iron, lead, and chromium in fish

According to the findings, in comparison to O. niloticus, C. gariepinus showed higher levels of the examined metals. Fish from the downstream study sites, on the other hand, accumulated larger levels of metals than those from the upstream study sites. The results also suggest that lead and chromium levels in both fish species' tissues were greater than the allowed values in fish, as shown in Table 7. The permitted limits for iron set by WHO (2011) and FAO (2011) are 43 mg/kg, 0.2 mg/kg for lead set by EU (2001), and 0.15 mg/kg for chromium set by WHO (1985) and FEPA (2003).

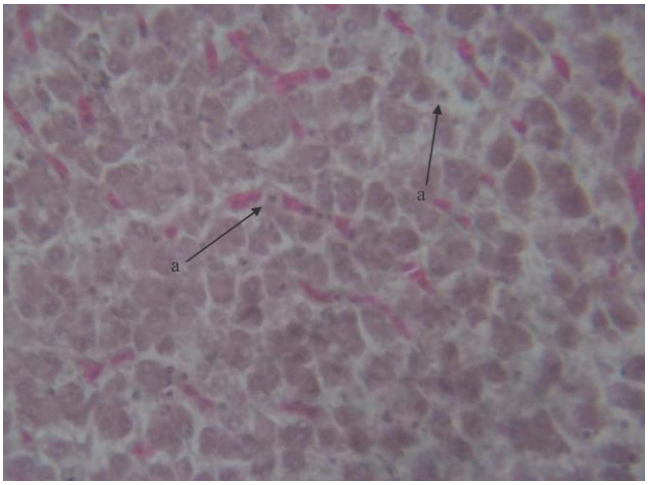

Figure 8. Liver of *Clarias gariepinus* obtained from the upstream study sites, showing normal hepatocytes with nuclei (a) (Haematoxylin and Eosin, × 400)

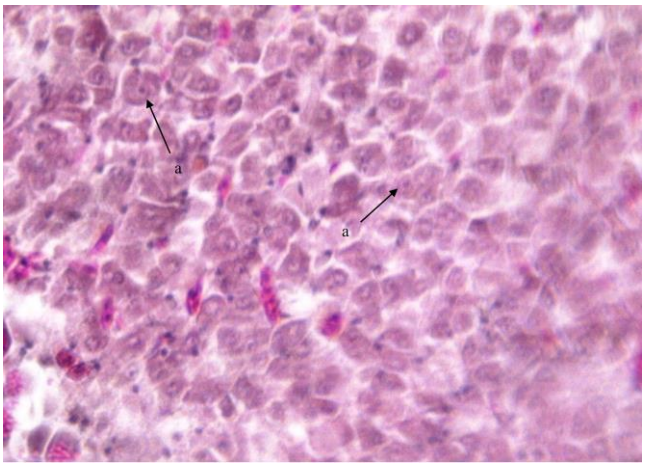

Figure 9. Liver of *Oreochromis niloticus* obtained from the upstream study sites, showing normal hepatocytes with nuclei (a) (Haematoxylin and Eosin, ×400).

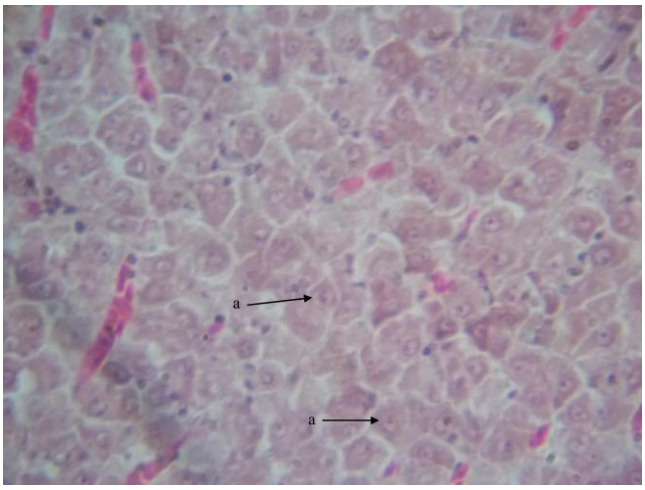

Figure 10. Liver of *Clarias gariepinus* obtained from the downstream study sites, showing enlarged hepatocytes (a) (Haematoxylin and Eosin, ×400).

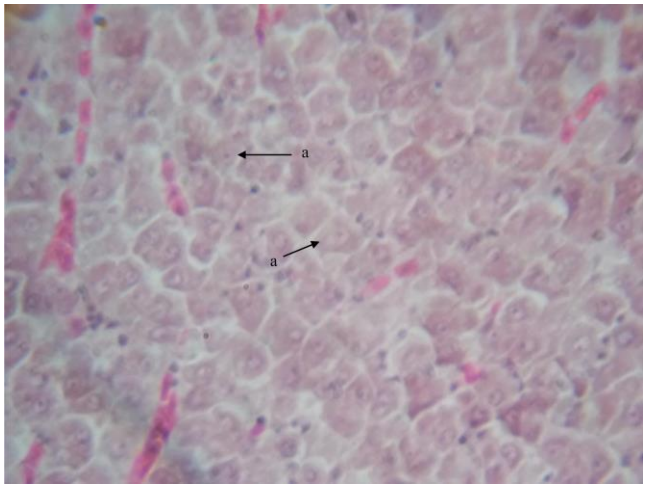

Figure 11. Liver of *Oreochromis niloticus* obtained from the downstream study sites, showing enlarged nuclei (a) (Haematoxylin and Eosin, × 400).

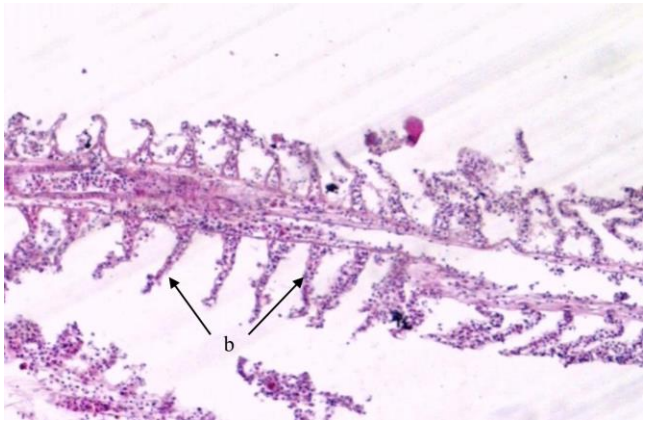

Figure 12. Gills of *Clarias gariepinus were* obtained from the upstream study sites, showing gill filaments and lamellae (b) (Haematoxylin and Eosin, × 150).

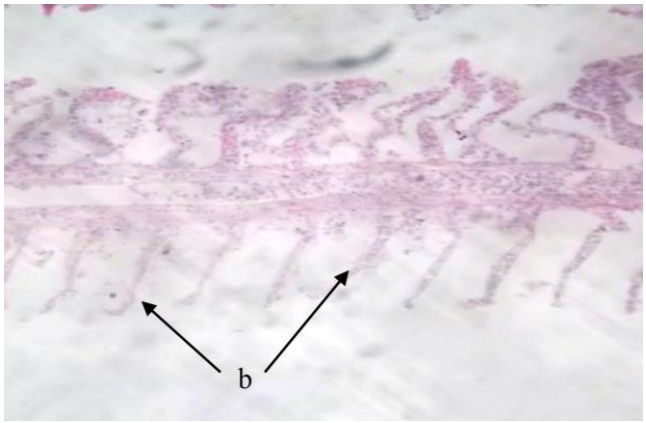

Figure 13. Gills of *Oreochromis niloticus were* obtained from the upstream study sites, showing gill filament and lamellae(b) (Haematoxylin and Eosin, × 150).

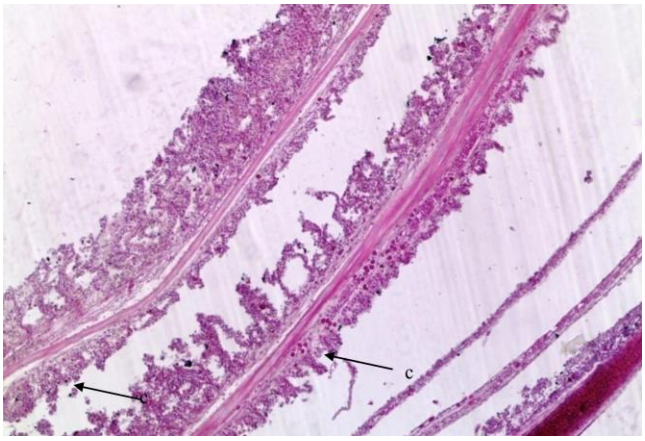

Figure 14. Gills of *Clarias gariepinus* were obtained from the downstream study sites, showing proliferation of interlamellar epithelial cells, which also show hyperplasia (c) (Haematoxylin and Eosin, × 150)

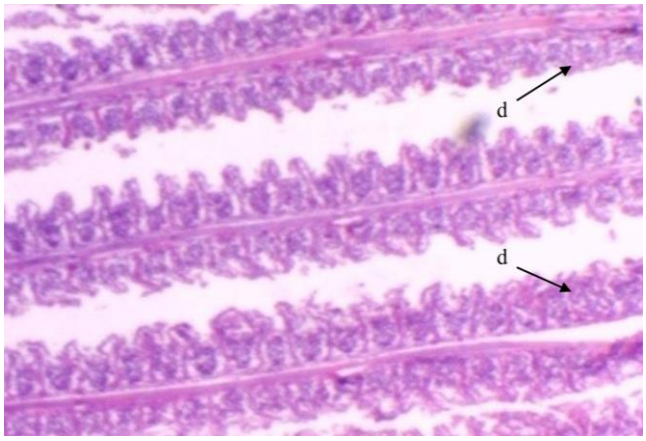

Figure 15. Gills of *Oreochromis niloticus were* obtained from the downstream study sites, showing fusion of secondary lamellae and hyperplasia due to increased multiplication of the epithelial cells lining the secondary lamellae and primary lamellae (d) (Haematoxylin and Eosin, × 150).

Table 7. Comparison of levels of iron, lead, and chromium in fish tissues, sediments, and water against permissible levels

Levels in mg/kg	Upstream study sites	Downstream study sites	P	Permissible values (mg/kg dry weight)	References
Iron in tissues of *Clarias gariepinus*	2.84±0.30	3.58±0.38	0.018	43	WHO 2011¸ FAO 2011
Iron in tissues of *Oreochromis niloticus*	1.98±0.20	2.44±0.25	0.011	43	
Lead in tissues of *Clarias gariepinus*	0.45±0.11	0.86±0.18	0.000	0.2	European Union 2001
Lead in tissues of *Oreochromis niloticus*	0.33±0.08	0.48±0.11	0.016	0.2	
Chromium in tissues of *Clarias gariepinus*	0.35±0.07	0.41±0.08	0.178	0.15	FEPA 2003
Chromium in tissues of *Oreochromis niloticus*	0.26±0.05	0.29±0.06	0.183	0.15	
Iron in sediments	0.58±0.16	0.62±0.18	0.112		
Iron in water (mg/L)	0.18±0.03	0.25±0.05	0.018	20	FEPA 1999
				0.2	NWQMS 2000
Lead in sediments	0.44±0.09	0.45±0.09	0.110	50	NWQMS 2000
Lead in water (mg/L)	0.12±0.03	0.22±0.05	0.059	0.1	FEPA 1999, 2006
				2	NWQMS 2000
Chromium in sediments	0.25±0.06	0.36±0.07	0.002	80	NWQMS 2000
Chromium in water (mg/L)	0.14±0.04	0.28±0.07	0.044	<1	FEPA 1999
				0.1	NWQMS 2000

Discussion

Levels of iron, lead, and chromium in sediments and water along Ruiru River

Higher levels of metals in sediments at downstream study sites than upstream study sites could be attributed to their role as reservoirs for pollutants coming from the surrounding areas and important sinks for various pollutants such as trace metals. Under the right conditions, they can also help with trace metal remobilization in aquatic systems (Aderinola et al., 2009; 2012; Uzairu et al., 2009; Osman et al., 2012). They increased total dissolved metal ions in water in the Ruiru River. Thus, an elevated level of metal ions that may have caused an increase in metals acquired can be linked to increased levels of iron, lead, and chromium in water and sediments observed from April to December. The elevated levels of chromium in water and sediments in December compared to April and August. The metals could have come from industrial, municipal, urban, and agricultural pollutants that runoff into the river. During wet seasons, lower levels of heavy metals in water may result from a dilution effect generated by additional water in the river due to rains (Damodharan and Vikram 2013). Organic substances from nearby farms may have contributed to the occurrence of heavy metals in the upstream study locations (Emere and Dibal 2013). Higher levels of metals in Ruiru River water during December, as observed in this study, could be linked to industrial effluents and human activities within Ruiru town, as well as evaporation of water due to increased temperatures associated with the dry spell, which typically begins in September and lasts through October and December, resulting in higher levels of metal residues in the river water. Significantly greater amounts of chromium documented in sediments at the downstream study sites and

iron, lead, and chromium in water at the same sites, p 0.05, can be attributable to higher temperatures at the downstream study sites over the three-month investigation (p 0.05). Warmer water speeds up the breakdown of wastes and the dissolution of chemicals in the water (Isyagi et al., 2009). As a result, greater temperatures at the downstream study sites along the Ruiru River may contribute to higher levels of the examined metals observed at these sites, as well as in the tissues of the fish found there. Furthermore, significant positive correlations between levels of iron, lead, and chromium in sediments and water samples collected from downstream study sites suggest that there was a strong tendency for high levels of metals in sediments to increase in tandem with increased levels of metals in water.

The proportional increase in temperature of water at downstream study sites along the Ruiru River has the potential to impact water's oxygen retention capacity, as temperature affects the quantities of dissolved oxygen in the water column, which is inversely related to temperature. Furthermore, the entry of surplus organic matter along the Ruiru River's downstream sections may result in a fall in oxygen levels, especially when the conditions are warm. Fish require oxygen for metabolism, and dissolved oxygen is required to convert potentially hazardous metabolic wastes into less toxic forms, such as ammonia (NH_3) to nitrite (NO_{2-}) and finally nitrate (NO_3) (NO_{3-}). The amount of dissolved oxygen in the water impacts the compounds in the water. Metals harden and precipitate out of water in the presence of oxygen. These metals dissolve into the water significantly more hazardous to aquatic species when there is no oxygen present (CWT 2004). Temperatures in the extreme top range make maintaining dissolved oxygen concentrations more difficult, according to research (Dennis et al., 2009).

Levels of iron, lead, and chromium in the liver, gills, and muscle of Clarias gariepius and Oreochromis niloticus.

Elevated amounts of these metals in sediments and river water have been correlated with the presence of iron, lead, and chromium in the liver, gills, and muscles of *C. gariepinus* and *O. niloticus*. Based on the current data, the presence of iron, lead, and chromium in the liver, gills, and muscles of fish can be attributed to high amounts of metals in river water. In addition to anthropogenic activities inside the Ruiru Municipality, higher levels of iron, lead, and chromium in the surface waters at sampling sites downstream and upstream in the river could be attributable to agricultural operations along the river banks and industrialization in Ruiru town. In this study, *C. gariepinus* obtained from both upstream and downstream sites along Ruiru River had higher iron, lead, and chromium levels in the gills, liver, and muscles than *O. niloticus*, albeit the differences in mean levels of metals in the fish tissues were not significant. As a result, metal accumulation in fish gills, liver, and muscle can be related to metal concentrations in water (Damodharan and Vikram 2013; Hmoud et al. 2013; Kpobari et al. 2013).

Statistically significant differences in the levels of chromium in sediments at the upstream and downstream study sites and iron and lead levels. Chromium in water at the two sites during the sampling occasions could contribute to differences in the levels of metals accumulated in the two fish species investigated, resulting in higher levels of metals in the tissues. Differential metabolic demand varies between species and is responsible for fish's metal accumulation variations. Even when the metal concentration is relatively low, chronic metal exposure leads to increased accumulation and enhanced toxicity in fish. Organisms exposed to pollutants develop mechanisms to sequester and excrete them, but chronic exposure over a long period leads to increased accumulation, allowing metals to reveal their toxic effects (Paulami and Samir 2012).

The lipid content of the tissue also determines metal levels in fish, but the distribution of metals in fish tissues is determined by the metabolic demands of a specific tissue (Paulami and Samir 2012). The fish environment and biological factors also influence metal excretion in fish, and the final level in fish is determined by the fish's ability to regulate the metal (Ishaq et al., 2011; Paulami and Samir, 2012).

This study revealed that the levels of iron, lead, and chromium in the liver and gills of fish were higher than in the muscles. The liver and gills acquire the most metals among fish organs, emphasizing their usefulness as bioindicators for studying heavy metal levels in general. At the same time, the muscles contain the least amount of heavy metals (Edem et al., 2009).

Lower levels of metals in the muscles of fish compared to the gills and liver reported in this study are comparable to earlier work by Ebenezer and Eremasi (2012), who found lower levels of copper, lead, cadmium, and nickel in the muscles of *Tilapia zilli* compared to its gills, and Javed and Usmani (2011), who found a similar order of heavy metal accumulation in the tissues of different fishes, with least accumulation of metals in the muscles. They also discovered that chromium was the metal with the least accumulation in all tissues investigated. Crafford and Avenant (2011) found greater levels of chromium, copper, iron, and manganese in the liver and gills of *C. gariepinus*, which was validated by the findings of this study.

Metal uptake also occurs through food; therefore, the metal accumulating in fish is influenced by their location, feeding habits, and trophic level. Most fish are at the top of the aquatic food chain and can acquire many metals even in low-pollution situations (Kpobari et al., 2013). In the current study, discrepancies in iron, lead, and chromium levels in *C. gariepinus* and *O. niloticus* might be associated with various factors, including feeding patterns, habitats, ecological demands, metabolism, biology, and fish physiology. The way fish are fed has a significant impact on the amount of heavy metals they accumulate (Ishaq et al., 2011). *O. niloticus* is adapted to feeding on a low trophic level and does not normally feed in deep waters (Robert 2001). They are primarily herbivorous and have a well-balanced diet, with healthy vegetable and animal components. Plankton, green leaves, benthic creatures, aquatic invertebrates, larval fish, debris, and decaying organic materials are all eaten by *O. niloticus*. Although

adult *O. niloticus* are not usually piscivorous, youngsters eat larval fish.

Clarias gariepinus, on the other hand, is euryphagous and is thought to be an opportunistic, omnivorous predator. *C. gariepinus* is primarily omnivorous, eating on detritus, invertebrates, and small fishes, and can transition between alternate food sources such as plants and detritus when prey animals become rare (FAO 2014). *C. gariepinus* is a bottom feeder by nature; however, they can modify their eating habits and filter feed in groups above the water surface occasionally. Because the fish has physical adaptations for piscivorous and filter-feeding (largemouth, marginal and pharyngeal teeth, robust and muscular stomach, and short intestine), it can shift from one feeding habit to the other on the availability and emergence of some food organisms (Elias 2009).

Compared to O. niloticus, C. gariepinus may consume mud-burrowing organisms to complement their diet, resulting in unintentional silt consumption and increased metal loading. Bottom-feeding fish come into more contact with sediments containing higher amounts of heavy metals; therefore, eating sediments while digging for food exposes the fish to additional metals. As a result, bottom-feeding fish have greater levels of heavy metals in their tissues (Ling et al., 2013). *O. niloticus* and *C. gariepinus* have different feeding patterns yet share the same habitat. The findings demonstrate that the investigated tissues of the omnivore fish *C. gariepinus* accumulated larger amounts of heavy metals than those found in the herbivorous fish *O. niloticus*.

The current study discovered positive correlations between iron, lead, and chromium levels in the fish liver, gills, muscles, and fish length and weight. The correlations, however, were not statistically significant. Heavy metal levels in fish tissues have also been shown in previous studies related to the fish's age and thus to the fish's size and length (Damodharan and Vikram 2013). Metals can accumulate in fish tissues faster than the metal's rate of excretion as the fish grows, and the accumulation is organ and species-specific (Indrajith et al., 2008). The relationship between tissue metal levels and fish size has not been studied in the Ruiru River fish population. Iron, lead, and chromium concentrations in the liver, gills, and muscles of *O. niloticus* and *C. gariepinus* were studied about the fish length and weight. According to the results, there was a positive correlation between metal concentrations and both fish length and weight. It has been found that metal accumulation in fish is stabilized at a certain age, indicating that the concentrations of metals are regulated and maintained at certain levels (Yi and Zhang 2012). However, suppose metal concentrations in the surrounding water are higher than the capacity of these factors. In that case, the dilution of tissue metal concentrations associated with growth and lowered metabolic activity in older individuals may not be seen. Here, metals may continue to build up, with a positive relationship between the size of the animals and metal concentrations in tissue (Yi and Zhang 2012).

Previous studies have shown positive and negative relationships between heavy metal levels in the tissues and fish length and weight. Usha and Vikram (2013) found that heavy metal levels in fish species decreased as fish size increased. In contrast, Canli and Atli (2003) found that zinc and lead accumulation in fish gills increased as fish size increased. That negative correlations between metal levels and size may be due to differences in metabolic activity between younger and older fish. A similar relationship has been observed between fish age, length, and weight and the accumulation of heavy metals in all tissues. Younger fish show higher nickel, lead, and chromium levels due to higher metabolic rates (Sahar et al., 2014).

As the length and weight of *O. niloticus* increase, the levels of copper, iron, lead, manganese, and zinc increase, while aluminum, cadmium, and mercury levels decrease (Authman 2008). According to research, zinc and lead levels in fish increase with body weight, particularly in the liver, gills, and kidneys (Paulami and Samir 2012). According to research, fish size and age are crucial characteristics when addressing metals accumulation in fish. Some elements' concentrations remain constant while others grow as fish size and age rose (Naeem et al. 2011). Luciobarbus xanthophores fish showed a positive relationship between heavy metal accumulation in muscle and size (Dusukcan et al., 2014). According to the researchers, there was a strong positive correlation between fish age and muscle heavy metal concentrations. There was a positive correlation between levels of metals in the studied fish tissues and both length and weight of fish, which could be an indication of possible acquisition and accumulation of metals in fish from the surrounding water, and could also point to the observed morphological changes that higher levels of metals present in the tissues of fish living in the affected areas downstream along Ruiru River.

Morphological effects of iron, lead, and chromium in liver and gills of C. gariepinus and O. niloticus.

There were structural differences between the liver and gill tissue of both C. gariepinus and O. niloticus caught from downstream and upstream study sites. There is a possibility that the metals present in the water are responsible for the observed morphological effects. In the current study, iron, lead, and chromium levels were significantly lower in the relatively unpolluted upstream study sites along Ruiru River than in the downstream study sites. In comparison to the downstream sections of the river, the upstream sections are comparatively free of pollution from industries, irrigation, and domestic effluents. Hepatocyte cytoplasm vacuolation, necrotic regions, and thickening of hepatocyte cells found in fish from downstream study sites along the river can be connected to toxic liver damage caused by river pollution. Heavy metals produced changes in the liver of *O. niloticus* living in the Nile River, according to Atif et al. (2009). Their histological study revealed several pathological alterations in the livers of *O. niloticus* living in the studied stations, including fatty degeneration, necrosis, and edema, and they explained that the discharge of various types of wastes, including heavy metals, degraded the water quality in the river, affecting aquatic inhabitants. Hepatocyte injury

was also shown in Deore and Wagh's (2012) study. They also discovered that the severity of damage depended on both the dose and the duration of metal exposure. Heavy metal salts enter cells by quickly crossing cell membranes, where they interfere with the cell's enzyme systems, causing morphological damage (Bhatkar 2011). In hepatocytes, heavy metals promote degeneration and vacuole necrosis, as well as hemolysis and hemosiderin pigmentation (Seham and Soad 2005). The accumulation of hemosiderin pigments in the liver tissue is caused by the rapid and ongoing destruction of erythrocytes due to increased hemolysis and disruption to the iron metabolism (Seham and Soad 2005). Large amounts of iron may be absorbed through the intestinal mucosa, resulting in aberrant hemosiderin buildup in the liver. Lysosomal membranes, which are particularly sensitive to numerous pathogenic stimuli, are disturbed, and their enzymes are released, resulting in liver cell degeneration and vacuolation (Seham and Soad 2005).

Previous studies reported histopathological changes happened in the muscles, liver, gills, kidneys, and intestine liver of *Tilapia zillii* and *Solea vulgaris* from Lake Qarun. They suggested that the pathological changes in the tissues of both studied fish could directly result from heavy metals, pesticides, fertilizers, salts, and sewage that enters the lake via the drainage water (Fatma 2009). The liver, the primary organ of metabolism, is constantly exposed to xenobiotics absorbed from the environment, and liver diseases are strongly associated with aquatic pollution (Fatma 2009). Hepatocyte degeneration, nuclear pycnosis, cellular edema, and blood vessel congestion were also seen in the livers of *O. niloticus* subjected to zinc (Abdel et al., 2011).

The histology of fish gills from the downstream parts of the Ruiru River revealed proliferation of the interlamellar epithelia, secondary lamellae degeneration, club-shaped primary lamellae, secondary lamellae hyperemia, and secondary lamellae fusion in this study. These impacts can be attributed to much greater metal levels recorded at these research sites than at the upstream study sites. Hexavalent chromium, which may easily pass through gill membranes and concentrate at higher amounts in various organs and tissues, can have a harmful effect both internally and on the gill surface. Chromium is particularly harmful since it may accumulate in many organisms at levels up to 4000 times higher than the ambient level (Avenant and Marx 2000).

The gills, which are involved in various critical processes in the fish, including breathing, osmoregulation, and excretion, stay in close contact with the water. They are sensitive to changes in water quality and are considered the pollutants' principal target (Jalaludeen and Arunachalam 2012). The response they elicit can range from almost non-existent to severe and widespread, resulting in lesions and tissue damage, and side effects like hyperplasia. Hyperplasia, fusion, and necrosis are the most prevalent types of lesions. Hyperplasia - aberrant increases in the amounts of cells in the gill epithelium – is the most common response to stimulation (Fitzroy Report 2012).

The gills regenerate uncontrollably due to continued deadly lead exposure, and the pillar cells become haphazardly placed. As a result, the space between adjacent secondary lamellae is nearly completely filled with polygonal epithelium, and the gill filaments appear as a solid mass of cells. The tips of adjacent main lamellae (PL) also fuse, leaving no gap between them. The secondary lamellae become multi-layered thick due to uncontrolled hyperplasia of the respiratory epithelium's polygonal cells. The secondary lamellae's free surface is wholly gone, and the secondary lamellae appear as a solid mass. By raising the blood oxygen barrier distance, the respiratory of fish efficiency is reduced. However, extending the diffusion distance of xenobiotics through the gill epithelium also slows xenobiotic penetration. Extracellular vacuolization between polygonal cells is common, and these polygonal cells appear to be metabolically active (Parashar and Banerjee 2002). The much greater levels of iron in water measured at the downstream study sites can also be linked to the morphological abnormalities found in the liver and gills of fish in this study. The presence of ferrous iron (Fe II) and the oxidation of ferrous to ferric (Fe III) iron are the main causes of iron toxicity. Furthermore, Fe (II) can induce tissue damage by forming free radicals and causing lipid peroxidation. Temperature and pH are significant iron modifiers toxicity, and a combination of low temperature and low pH can result in relatively high levels of poisonous Fe (II) (Nicolas and Martin 2003). Fish can be poisoned by the transition of iron from Fe (II) to Fe (III) and the creation of Fe (III) precipitates. The production of solid, or colloidal, precipitates is related to a decrease in Fe (II) and an increase in Fe (III), and a change from low molecular mass forms to higher molecular mass forms of iron. These changes in iron behavior are accompanied by a rise in gill iron concentrations (Adam et al., 2011).

Dilation and wall thickening of blood vessels in the liver, which becomes clogged with blood cells, cytoplasmic vacuolation of hepatocytes with profoundly colored nuclei, and cytoplasmic vacuolation of hepatocytes with deeply stained nuclei are all observed in tilapia treated with lead nitrate (Bothaina et al. 2012). During ten days of exposure, the toxic effects of chromium on the histology of gill and liver of *Labeo rohita* fingerlings cause modest histological changes. After thirty days, however, gill lamellae fusion, hypertrophy, and epithelial degradation are visible (Muthukumaravel and Rajaraman 2013). Vacuolation, hepatocyte degeneration, and hepatocyte cell boundary disintegration are examples of liver pathologies caused by metal exposure. Because the liver is the primary organ for detoxifying, changes in the liver may be linked to the direct harmful effects of contaminants on hepatocytes (Mohammad et al., 2013). Chromium, nickel, and zinc chlorides cause degenerative histopathological changes in Labeo rohita's liver, including larger nuclei, condensation of cytoplasm, and hepatic cords' disorder and blood congestion in sinusoids, vacuolation of hepatocytes, and necrosis (Bhatkar 2011).

Higher chromium levels in the water at the downstream study sites along the river may have contributed to the structural changes seen in the fish. The effects of chromium in the gills of *Cyprinus carpio* were dosage dependant, with disintegrating gill mucosal epithelium, basement membrane, and submucosa cells shown at lower doses and

hyperplasia seen at higher doses (Solangi et al. 2012). According to the researchers, disintegrated primary and secondary lamellae were also detected at lower chromium concentrations, followed by hyperplasia at higher concentrations. Total mean levels of iron, lead, and chromium in fish

Iron had the highest levels of metals in the tissues of the fish species investigated, whereas chromium had the lowest. Compared to *O. niloticus*, *C. gariepinus* had consistently high amounts of iron, lead, and chromium. The liver of *C. gariepinus* has the highest amounts of all metals. Ekpo et al. (2013) found high amounts of zinc, manganese, copper, lead, cadmium, and chromium in the liver and gills of *Heterotis niloticus*, *O. niloticus*, and *C. gariepinus* collected from Akampa Local Government Area, while muscles accumulated the least quantities of metals.

The liver is a detoxifying organ that is responsible for the metabolism and excretion of toxins in the body. All hazardous chemicals are likely to be processed in this organ because it is the center of metabolic processes (Ali and Shaakori 2011; Ekpo et al. 2013). The liver is prone to toxins because of its location and proximity to the venous drainage of the digestive tract; the high metabolic activity of hepatocytes makes them vulnerable, and toxins can quickly affect them (Yousuf et al., 2013). Higher levels of metals in the liver have also been linked to the fact that it is an organ most connected with detoxification and biotransformation processes, as well as one of the organs most affected by toxins in the water due to its function, position, and blood supply (Fatma 2009). Metal levels in fish tissues were found to be higher than those set for fish by international regulations and guidelines such as EC (2001), WHO (2011), FEPA (2003, 1999, and 2006), and FAO (2001). (2011). Metals in the tissues of *C. gariepinus* and *O. niloticus* observed in the current study surpassed the permitted limits defined for heavy metals.

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Isolation and identification of phosphate solubilizing bacteria from the rhizosphere of rice in organic and non-organic rice fields in Sukoharjo District, Indonesia

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Abstract. *Purwanti D, Setyaningsih R, Susilowati A. 2019. Isolation and identification of phosphate solubilizing bacteria from the rhizosphere of rice in organic and non-organic rice fields in Sukoharjo District, Indonesia. Bonorowo Wetlands 9: 108-115.* One of the essential nutrients in the soil is phosphate. Phosphate fertilization is often inefficient because phosphate is bound and difficult for plants to absorb. The use of phosphate solubilizing bacteria (PSB) can be used as an alternative to the availability of P in the soil. This study aimed to obtain PSB isolates with high phosphate solubilization ability and identify these PSB isolates molecularly. Soil samples were taken from organic and non-organic rice (*Oryza sativa* L.) fields in Sukoharjo, Central Java, Indonesia, namely Grogol Village, Menuran Village, and Pondok Village. The research activity was carried out in several stages: isolation of PSB on Pikovskaya medium, characterization and testing of PSB in dissolving P, and Gram staining. PSB colonies with a phosphate solubilization index and showed different colony colors were taken, and then DNA was isolated. DNA isolation was carried out by extraction method using a DNA extraction kit. The gene encoding 16S rRNA amplified the obtained DNA samples. Sequencing was carried out using a genetic analyzer. Data analysis by comparing sequence data on the BLASTN program. Based on the isolation of rhizosphere samples from organic paddy fields, 14 isolates of PSB were obtained, and ten isolates of non-organic paddy were PSB. P13, P1, Q7, and Q8 isolates had the highest P solubilization ability with dissolution index (IP), namely 3.35, 2.13, 1.82, and 1.47. The 16S rRNA gene sequence analysis showed that isolates P1 and P13 had 99% similarity to *Acinetobacter* sp. ADP1 strain. The isolate Q7 had a 96% similarity with the genus *Clavibacter* strain NCPPB 382, while Q8 had a 99% similarity with *Pseudomonas aeruginosa* strain PAO1.

Keywords: Gene encoding 16S rRNA, organic and non-organic rice fields, phosphate, phosphate solubilizing bacteria

INTRODUCTION

Indonesia is an agricultural country with a broad agricultural sector and has a vital role in supporting the national economy. According to Simamora (2006), challenges in the agricultural sector will continue to increase. It is estimated that by 2025 the population growth will reach 8.5 billion, and most of them will come from developing countries. Technology and agricultural innovation are needed to support increased food production, especially rice.

Fertilizer is a material used to change soil's physical, chemical, and biological properties to improve plant growth. Chemical fertilizers played an essential role during the green revolution, but the imbalance reduced soil fertility and environmental damage (Gyaneshwar et al. 2002). Thus, humans are aware of the negative impact of chemical fertilizers on the environment, thereby encouraging the development of organic farming systems (Andoko 2008). Organic agriculture avoids chemicals, including chemical fertilizers, that are toxic to the environment to obtain a healthy environment (Sutanto 2002; Andoko 2008).

In organic farming, the fertilizers used are organic fertilizers and biological fertilizers. Organic fertilizers are fertilizers in which all organic materials come from animals or plants, while biological fertilizers are derived from all functional groups of soil microbes. The advantages of biological/organic fertilizers in implementing organic farming are that they are more environmentally friendly than chemical fertilizers, can increase land productivity, and preserve the environment (Suriadikarta and Simanungkalit 2006). Biofertilizers also leave no residue on crop yields, so they are safe for human health (Musnamar 2003).

One element that is often given during fertilization is phosphorus. Phosphorus is an element that is needed in large amounts (macronutrients). Plants absorb phosphorus in primary orthophosphate ions (H_2PO_4-) and secondary orthophosphate ions (HPO_4^{2-}). Generally, P is not entirely soluble in water because P can react with other ions and form compounds whose solubility is reduced to become compounds that are not easily washed. Most of them become compounds unavailable to plants (Rosmarkam and Nasih 2002).

Most of the paddy fields in Indonesia are saturated with phosphate due to the high use of chemical fertilizers. Still, plants cannot absorb the phosphate because it is bound to other compounds, so farmers apply phosphate (P) fertilization to increase the dose, even though the amount of phosphate in the soil is quite large. The phosphate-binding with soil colloids causes phosphate fertilization to

be less efficient and can cause soil pollution (Kurnia et al. 2008). P deficiency can cause the volume of plant tissue to be smaller and the leaf color to be darker. One of the efforts to overcome the availability of P in the soil is to utilize microorganisms (Rosmarkam and Nasih 2002).

Phosphate fertilization efficiency can be done by utilizing phosphate solubilizing bacteria. Phosphate solubilizing bacteria can provide plants with phosphate not previously available in the soil through conversion. The mechanism of phosphate solubilizing bacteria in dissolving inorganic P is by excreting organic acids due to bacterial activity in the rhizosphere (Hardjowigeno and Rayes 2005).

Phosphate solubilizing bacteria can be isolated from around plant roots. The ability of bacteria to dissolve phosphate can be tested by growing it on Pikovskaya media containing Ca_3PO_4. The dissolution of Ca, which binds P in Pikovskaya media, can be seen from the width of the clear zone formed around the colony. The larger the clear zone, the greater its ability to dissolve phosphate. The increase in the width of the clear zone, which was higher by one isolate against another, showed an indication that this bacterial isolate had superior characteristics. An increase also follows the increase in the clear zone in the diameter of the bacterial colony (Maryanti 2006).

In general, studying bacteria's morphology, cell structure, and biochemical properties is done by isolation. Bacterial characterization can be done by identifying 16S rRNA (Reeve 1994). The 16S rRNA gene is relatively constant and does not change for a very long time; in other words, the mutation rate is minimal, so it is relevant when used as an object of research (Janda and Abbott 2007).

The aims of this study were (i) to obtain isolates of phosphate solubilizing bacteria from organic and non-organic paddy soils that have high phosphate solubilization ability in Sukoharjo and (ii) to obtain the names of bacterial species that have a high ability to solubilize phosphate from organic and inorganic paddy soils based on the 16S rRNA gene sequence.

MATERIALS AND METHODS

Ingredient

The main ingredients were soil samples from organic and non-organic rice rhizosphere from three villages in Sukoharjo District, Central Java, Indonesia, namely Grogol, Menuran, and Pondok villages. Pondok village farmers are the pioneers of organic farming in Sukoharjo. Most of the productive/working-age population, especially those aged between 15-50 years, work as farmers in the food agriculture sector. They have carried out the development of organic rice farming, and all farmers have carried out organic rice farming, both those who have followed all the stages or only some of the stages (Rustiono 2008).

Procedures

Rhizosphere sampling

Rice rhizosphere was taken from organic and non-organic farmland in the Sukoharjo area. The roots of the rice plants are cut using scissors to separate them from the stems, then shaken. The collected soil is still attached to rice roots (*Oryza sativa* L.). The rhizosphere sample was then put into a sterile bottle (Prasanna et al., 2011).

Pikovskaya medium production

A total of 0.5 grams of yeast extract, 10 grams of dextrose, 5 grams of $CA_3(PO_4)_2$, 0.5 grams $(NH_4)_2SO_4$, 0.20 grams of KCL, 0.1 grams of $MgSO_4.7H_2O$, 0.0001 grams of $MnSO_4.H_2O$, 0.0001 grams $FeSO_4.7H_2O$, 18 grams agar homogenized in 1000 mL distilled water. Medium ph 6.8 and autoclave sterilization for 20 minutes at 121°C (Prasanna et al. 2011).

Phosphate solubilizing bacteria

Isolation of phosphate solubilizing bacteria (PSB) and purification of isolates were based on the method of Prasanna et al. (2011). The soil from each sample was put into a sterile 250 mL Erlenmeyer with a size of 10 grams and homogenized with 90 mL of sterile distilled water in an orbital shaker (200 rpm, 30 minutes). The sample was left for 10 minutes. A total of one mL of the sample supernatant was put into a flask that already contained nine mL of 0.85% NaCl, then put into 6 test tubes with each dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. Furthermore, 0.1 mL of each dilution was spread in a petri dish filled with Pikovskaya medium. Anaerobic incubation at 28°C ± 2 to 7 days in an incubator. In a petri dish, bacterial colonies will grow. Colonies surrounded by a clear zone indicate the presence of phosphate dissolution. Colonies with a wide clear zone and different colony colors were taken to dissolve phosphate qualitatively. The colonies were counted and expressed in colony-forming units (CFU) per gram of soil, then phosphate solubilizing bacteria were identified.

Clear zone measurement

The growth of phosphate solubilizing microbial colonies in Petri dishes from the culture results was observed using a magnifying glass. Then the colony diameter and clear zone diameter were measured with a ruler. The colony diameter and clear zone were measured 2-3 times at different positions, and the measurement results were averaged. The way to measure it is:

$$\text{Phosphate Solubility Index} = \frac{\text{diameter of clear zone - diameter of colony}}{\text{colony diameter}}$$

Colonies with high ratio values were isolates of phosphate solubilizing bacteria taken for identification (Saraswati et al. 2007).

Bacterial colony and cell morphology characterization

The bacterial isolates were identified for their cell morphology by using the Gram stain test and microscopic observation of the shape of the microbe. Gram staining is done by passing a glass object over a Bunsen flame. Then one drop of sterile distilled water was dropped on a glass object, followed by pure isolate. The isolates and aquadest were flattened and then dried and fixed. The surface was

dripped with crystal violet solution and left for 30-60 seconds, and then was washed with running water and dried. Iodine solution is dripped on the surface of the preparation, left for 30-60 seconds, washed with running water, and dried. Next, the preparations were washed with 96% alcohol for 5 seconds, then dripped with safranin and left for 30-60 seconds. Finally, the preparations were washed with running water and observed under a microscope to see (color, shape, surface, edge, size, and optical characteristics) and the microscopic character of the colony in the form of Gram reaction and bacterial cell shape (Prasanna et al. 2011). If the staining results of bacterial cells were red, the cells were Gram-negative, while if they were purple, they were Gram-positive.

DNA extraction

DNA extraction using the GeneJET Genomic DNA Purification kit followed the procedure recommended by the manufacturer with the following steps: 1 mL of the bacterial isolate from 10 mL of previously cultured bacterial culture was put into a tube, then centrifuged at 3,500 rpm; 10 minutes. The supernatant was discarded. 180 µL Digestion Solution was added to the tube. Furthermore, a micropipette mixed 20 µL of Proteinase K Solution by vortexing/resuspension. Samples were incubated in a shaking incubator for ±30 minutes; 100rpm; 56ºC. Then 20 µL of RNase A Solution was added to the tube and suspended using a micropipette. Incubated at room temperature for 10 minutes. 200 µL of Lysis Solution was also added and resuspended using a vortex for 15 seconds until the mixture was homogeneous. Then 400 µL of 50% ethanol was added and resuspended. The resulting lysates were transferred to the GeneJET Genomic DNA Purification Column (ban tube). Centrifuge 4200 rpm; 1 minute. Discard the centrifuged liquid's collection tube and replace it with a 2 mL collection tube. Added 500 l Wash Buffer I (if the Kit used is new, it is necessary to add ethanol according to the manufacturer's procedure). Centrifuge 5600 rpm; 1 minute. Discarded the liquid that fell to the bottom of the tube. Also added 500 µL Wash Buffer II (if the Kit used is new, it is necessary to add ethanol according to the manufacturer's procedure) on the membrane tube. Centrifuge 8,400 rpm; 3 minutes. Optional: if the residue is still visible on the membrane tube, empty the collection tube and centrifuge to full speed. The collection tube containing the fluid was discarded, and the GeneJET Genomic DNA was transferred to a sterile 1.5 mL collection tube. Then 200 µL of Elution Buffer was added to the center of the GeneJET Genomic DNA Purification Column to elute Genomic DNA. Incubation for 2 minutes at room temperature and centrifuge 5,600 rpm; 1 minute. The purification column (membrane tube) is discarded, and the extract can be used immediately, or the extracted DNA can be stored at -20ºC

16S rRNA gene amplification

The amplification of the gene encoding 16S rRNA was carried out with primers 63F (5'CAG GCC TAA CAC ATG CAA GTC) and 1387R (5'GGG CGG WTG GTA CAA GGC) (Marchesi et al. 1998) with the following reaction mixture: DNA 0.1 g, 1x buffer, two µL 10 mM dNTP Mix, two U Taq DNA Polymerase, five pmol each primer, ddH2O to 25 µL. The PCR program consisted of initial denaturation at 95ºC for five minutes, followed by 30 cycles at 95ºC for 30 seconds, 55ºC for one minute, 72ºC for one minute, and then final extension at 72ºC for five minutes. The PCR results were observed by electrophoresis using 1% of agarose gel and 1x TAE buffer.

16S rRNA coding gene sequencing

Detection of nucleotide sequences (sequencing) using the ABIprism™ 310 Automated DNA Sequencer (PE Applied Biosystem), then repeated PCR for the sequencing stage (PT Genetics Science Indonesia – First Base, Singapore).

Data analysis

Sequence data obtained from the selection of isolates with the widest clear zone were then compared with sequences in the National Center for Biotechnology Information (NCBI) data bank in the Basic Local Alignment Search Tool for Nucleotides (BLASTN) program (http://www.ncbi.nlm.gov/BLAST/).

RESULTS AND DISCUSSION

Soil samples from the rhizosphere of rice plants (*Oryza sativa*)

Soil samples that were taken have the potential to develop organic agriculture. Organic farming in the three villages has been certified by an Organic Certification Institute that KAN has accredited. The definition of organic agriculture in the village is agriculture whose processing is done organically, namely by using organic fertilizers. Organic fertilizers used with the composition are as follows: manure (cow), compost, dolomite, husk charcoal, molasses water, and decomposing bacteria. In addition, irrigation in organic rice fields is different from non-organic rice fields, but the water source used is the same, namely springs from Mount Lawu. The irrigation system is treated differently in organic rice fields by making water reservoirs. The reservoir is given water hyacinth to neutralize water that still contains residues of chemical substances. Some organic rice fields do not use dams but make ponds in irrigation channels along with these fields. The pond is filled with water hyacinth and fish, holding water and neutralizing chemical residues. Fish in these ponds are usually sensitive to chemicals, intending to measure the level of chemical residues; if the fish in the pond die, the water may contain high chemical substances. Irrigation of non-organic rice fields is not given special treatment, so it is possible that the water still carries many chemical residues.

Phosphate solubilizing bacteria are often associated with organic and inorganic soil. In the soil, there are plant roots that microbes can utilize as nutrients in the form of exudate released by plants so that bacteria will associate in the plant rhizosphere. Fiantis (2012) said that organic soil

comes from plant remains that accumulate in an area. Conventional methods of non-organic soil are usually carried out for narrow land and have a particular slope. Thus, applying an organic farming system from the point of view of the availability of P nutrients is suitable to implement because it can increase the productivity of rice plants.

Phosphate solubilizing bacteria

Phosphate solubilizing bacteria (PSB) is one type of biological fertilizer that can make inorganic P fertilizers more efficient to overcome the low P-available soil and increase the P concentration of plants. The ability of PSB varies greatly depending on the type of microbe, adaptability, to the ability to produce organic acids and enzymes (Whitelaw 2000).

Colonies with morphological differences, including color, shape, and edges, were taken from all bacterial colonies that grew in each dilution. The entire colony was then purified again by streaking on the surface of the NA medium. Isolates that showed the presence of a clear zone were taken and then grown on Pikovskaya media as a solid test, and the phosphate solubilization index (SI) was measured. The results of observations of colonies with morphological differences (color, shape, and edges) can be seen in Table 2.

Twenty-four (24) bacterial colonies were obtained based on the selection, namely 14 bacterial colonies from organic fields and ten bacterial colonies from non-organic fields. The isolation of phosphate solubilizing bacteria in organic soils was more than in non-organic soils. In addition, bacteria in organic rice fields can dissolve phosphate higher than bacteria in non-organic rice fields. According to Suriadikarta and Simanungkalit (2006), phosphate solubilizing microbes are related to the amount of organic matter contained in the soil.

Twenty-four colonies were streaked onto the surface of the Pikovskaya medium containing 1% Ca_3PO_4. The colonies that formed the clear zone were purified by streak for a single colony on Pikovskaya media. These bacterial colonies that formed a clear zone around them were 20 PSB isolates. The PSB isolates were then re-grown on Pikovskaya media to determine the clear zone produced from each isolate. The diameter of this clear zone is used to calculate the phosphate solubilization index (SI). PSB with SI 1 was taken for further identification.

PSB produces acid during growth. According to Maryanti (2006), the signs that a bacterium can dissolve phosphate are the presence of a clear zone around the bacterial colony and an increase in the size of the bacterial colony on Pikovskaya media; this is because the bacteria can dissolve phosphate ($Ca_3(PO_4)_2$) contained in the Pikovskaya media formulation. The formation of a clear zone indicates that the bacteria can produce organic acids. The solid test results on Pikovskaya media were the 20 isolates with the highest SI with codes P1 and P13 from samples of organic rice fields and Q7 and Q8 from samples of non-organic rice fields (Figure 1).

Based on the resulting SI, it can be seen that PSB has various P-dissolving abilities (Table 1). According to Rachmiati (1995), the clear zone area qualitatively indicates the size of the bacteria's ability to dissolve phosphate. In the research conducted by Nopparat et al. (2007), P solubilizing bacteria and fungi have different abilities depending on the strain type. Superior P solubilizing microorganisms will produce the largest clear strain zone diameter compared to other colonies.

Characteristics of phosphate solubilizing bacteria isolates

Bacterial isolates obtained in the solid test in Pikovskaya media were then carried out in the Gram stain test. The results of the Gram staining test can be seen in Table 3.

Table 1. Characteristics of phosphate solubilizing bacterial colonies in dissolving p at solid Pikovskaya medium

Isolate code	Shape	Color	edge	Solubilization Index (SI)
P1	Light yellow	Round	Flat	2.13
P2	White	Round	Flat	-
P3	Yellow	Irregular	Curvy	-
P4	Yellowish white	Round	Flat	0.58
P5	Yellow	Long	Flat	0.41
P6	Milky white	Round	Flat	0.22
P7	Milky white	Round	Flat	-
P8	Yellow	Round	Flat	-
P9	Milky white	Round	Jagged	0.33
P10	Light yellow	Irregular	Flat	0.17
P11	Yellowish-white	Irregular	Curvy	0.17
P12	Yellowish white	Round	Flat	0.24
P13	Yellowish-white	Round	Flat	3.35
P14	Yellowish white	Irregular	Curvy	0.43
Q1	Milky white	Irregular	Curvy	0.73
Q2	Milky white	Round	Curvy	0.22
Q3	Milky white	Round	Flat	0.93
Q4	Orange	Irregular	Flat	0.39
Q5	Milky white	Irregular	Wavy	0.35
Q6	Milky white	Round	Flat	0.46
Q7	Yellowish white	Round	Flat	1.82
Q8	Light yellow	Round	Flat	1.47
Q9	Yellowish white	Round	Flat	0.41
Q10	Light yellow	Round	Flat	0.14

Note: PSB isolates from organic rice fields, PSB isolates from non-organic rice fields

Table 2. Gram staining of bacterial isolates isolated from the rhizosphere of rice (*Oryza sativa*) in organic and non-organic rice fields in Sukoharjo, Central Java, Indonesia

Isolate code	Gram	Cell shape
P1	-	single rod
P13	-	single rod
Q7	-	single rod
Q8	-	single cocci

The four bacterial isolates showed a red color after Gram staining. It indicates that the four bacterial isolates were Gram-negative bacteria. According to Purwani et al. (2009), Gram-negative bacteria have a relatively more complex cell wall structure, i.e., three layers of the cell wall. They were the outer layer in the form of lipoprotein, the middle layer in the form of lipopolysaccharide, and the inner layer in the form of peptidoglycan. Gram-negative bacteria whose lipid substances will dissolve during washing with alcohol. It causes the pores in the cell wall to enlarge, the permeability of the cell wall becomes large so that the dye that has been absorbed is quickly released, and the bacterial cell becomes colorless (pink). In Gram-negative bacteria, the red color of safranin is the outer membrane. Gram-positive, stained purple or blue by crystal-violet is the peptidoglycan layer. Gram staining was also carried out to determine the cell shape of PSB. Of the four bacterial isolates, three had a single rod form, and one had a single cocci form (Figure 2).

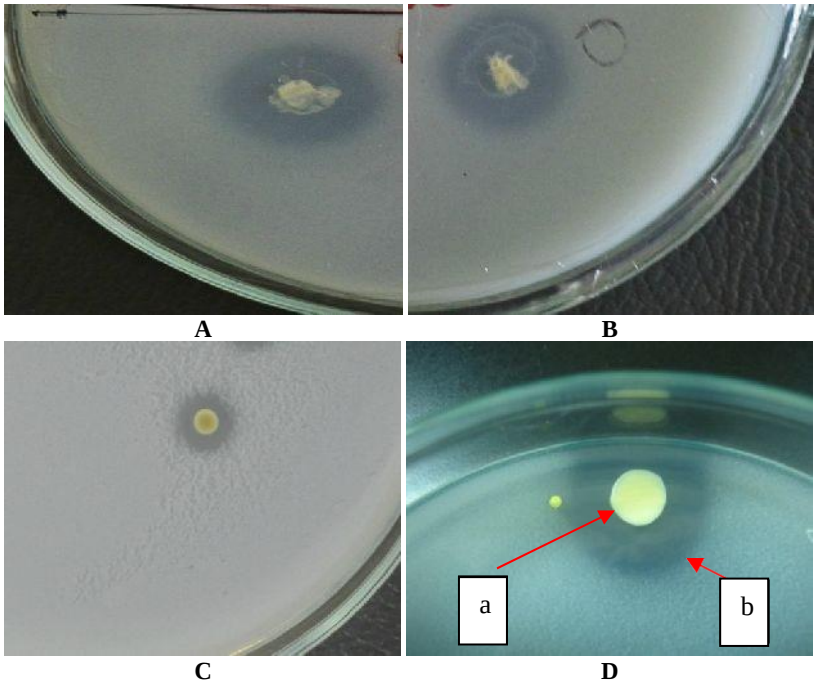


Figure 1. Clear zone by PSB isolates on Pikovskaya media. A. Isolate Q7, B. Isolate Q8, C. Isolate P1, D. Isolate P13. Sections marked with arrows: a. PSB isolates, b. clear zones

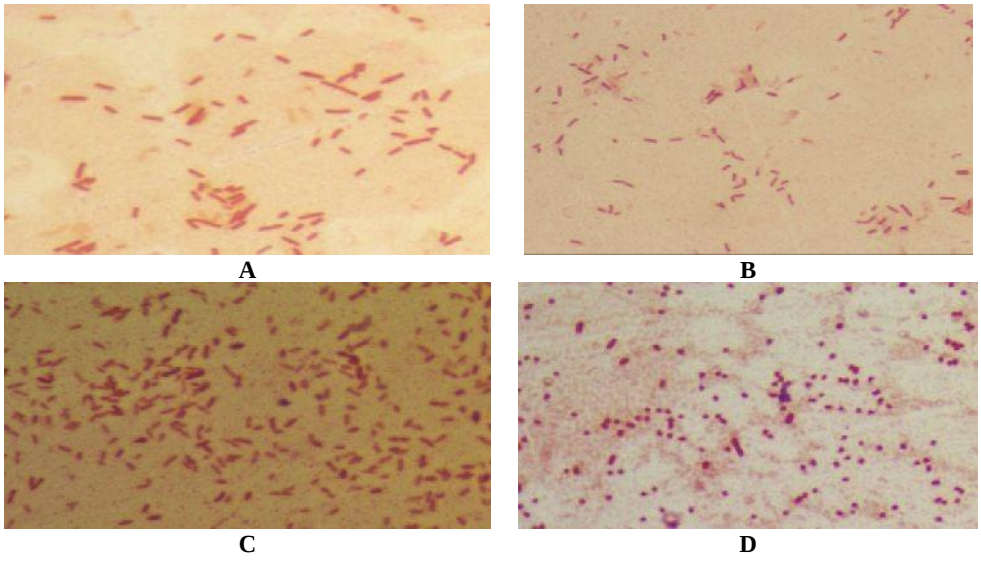


Figure 2. Gram stain test results on four bacterial isolates from the rhizosphere of rice plants (*Oryza sativa*). A. Isolate P1, B. Isolate P13, C. Isolate Q7, and D. Isolate Q8

Gene encoding 16S rRNA

The four bacterial isolates with the most potency to dissolve phosphate, namely isolates P1, P13, Q7, and Q8, were identified using the gene encoding 16S rRNA. The PCR product fragment has a size of about 1500 basepair (bp) which is the expected size using a primer combination of 63F (5' CAG GCC TAA CAC ATG CAA GTC3 3') for the *forward direction*, and 1387R (5' GGG CGG WGT GTA CAA GGC 3') for directions *reverse* (Marchesi et al. 1998). Primer is an essential component in the PCR reaction because this primer will determine the target region of the genome to be amplified. The electrophoresis results of PCR amplification of the 16S rRNA region of phosphate solubilizing bacteria are shown in Figure 3.

The electropherogram results showed that the 16S rRNA region of phosphate solubilizing bacteria was about 1300 bp in size. A single band was seen, indicating that the primer pair was specific to the desired region. The single band contains a collection of genes encoding 16S rRNA from bacterial isolates. The thickness and thinness of the bands indicate the quantity of amplified DNA. Electropherogram amplification of the genomic DNA of phosphate solubilizing bacteria in this study is clear and thick, indicating that the concentration of separated molecules in this region is high.

Phosphate solubilizing bacteria identity

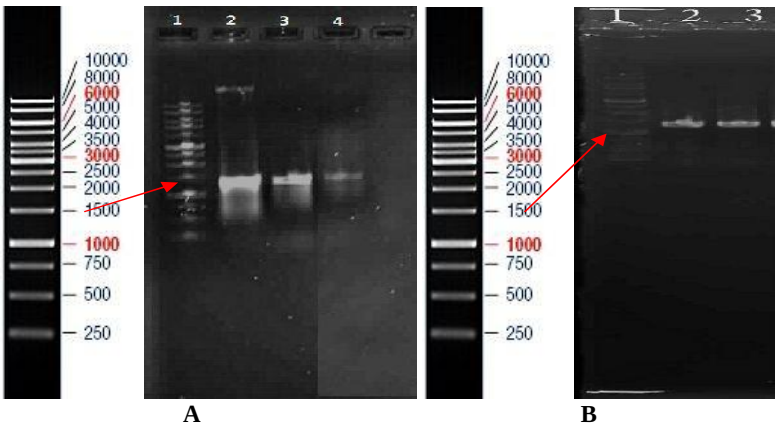
Nucleotide base sequences were analyzed and compared with the gene database from GenBank DNA using the BLAST program. Stephen et al. (1990) mentioned that the BLAST program was used to determine the identity and level of homology with previously known gene fragments.

Based on the results of BLASTn analysis, there are three sequences of phosphate solubilizing bacteria which have partial sequences similar to the data from GenBank with a similarity level of 99% (Table 3). It indicates that the bacteria are in the same species as the isolates that have been identified previously by Drancourt et al. (2000). The analysis results also showed that one sequence of phosphate solubilizing bacteria had a similarity value of <97%, meaning that the isolate was a new species for which data were not available in GenBank. The percentage of similarity <97% of the 16S rRNA gene fragment from the sequence indicated that the identified bacteria had a close resemblance to bacteria from the GenBank data but were not bacterial species from the GenBank data. Drancourt et al. (2000) stated that isolates with a 16S rDNA sequence similarity of 99% could represent the same species. Isolates with a sequence similarity of 97% could represent identity at the genus level.

Based on the results of the BLASTn analysis, it was found that the bacterial isolates in the organic rice field samples were *Acinetobacter* sp. while in non-organic rice fields, namely *Clavibacter* and *Pseudomonas aeruginosa*. *Acinetobacter* sp. is a bacterium of the same genus, namely *Acinetobacter*, a Gram-negative bacterium that grows optimally at a temperature of 33-35°C, stems in the form of stem cells, often found in soil and water (Doughari 2011). Bacteria of this genus can utilize glucose, mannitol, maltose, and sucrose by oxidation (Barrow et al. 1993). Pramono (1994) revealed that phosphate solubilizing bacteria create organic acids by glucose catabolism in the tricarboxylic acid (TCA) cycle, which continues glycolysis. These organic acids are primary metabolites used for cell survival so that the phosphate dissolution mechanism is more straightforward and can be absorbed by plants (Rodrigues et al. 2006).

Table 3. The percentage of DNA similarity encoding the 16S rRNA gene of phosphate solubilizing bacteria from the rhizosphere of rice plants in organic and non-organic rice fields with GenBank sequences

Isolate	Best friend	Access no.	Query cover (%)	% Similarity
P1	*Acinetobacter sp.* ADP1. strains	NC005966.1	97	99
P13	*Acinetobacter sp.* ADP1. strains	NC005966.1	98	99
Q7	*Clavibacter* strain NCPPB 382	NC009480.1	99	96
Q8	*Pseudomonas aeruginosa* strain_PAO1	NC002516.2	99	99



A **B**

Figure 3. Electropherogram of the 16S rRNA encoding gene amplicons. A. lane one marker, lane two bacterial isolates Q8, lane three isolates bacteria P13, and lane four isolates bacteria Q7; B. lane one marker, two and three isolates of P1 bacteria

According to Bergey's manual, *P. aeruginosa* includes aerobic rods and cocci, Gram-negative bacteria, which are motile because they have flagella, and some are non-motile (Pelczar and Chan 2006). These bacteria grow optimally at 37ºC (Stover et al. 2000). Bacteria of the genus *Pseudomonas* can produce catalase enzymes (Barrow et al. 1993). Besides that, *Pseudomonas* has been widely studied as a biocontrol agent because of its ability to produce antimicrobial metabolites. The phosphate solubilizing bacteria (PSB), *P. aeruginosa*, and *Bacillus* sp. were evaluated for antibiotic resistance to various antibiotics, including ampicillin, chloramphenicol, penicillin, and streptomycin at concentrations of up to 400 µg/mL medium (Setiawati 2000). According to Purwaningsih (2003), bacteria that act as phosphate solubilizers in soil have been found, including the genera *Pseudomonas*, *Micrococcus*, *Bacillus*, *Azotobacter*, *Microbacterium*, and *Flavobacterium*. The research results by Widiawati and Suliasih (2006) also stated that *Pseudomonas* and *Bacillus* bacteria were phosphate solubilizing bacteria that had the most remarkable ability as biofertilizers. It is done by dissolving phosphate elements bound to other elements (Fe, Al, Ca, and Mg) to make the P elements available to plants.

Continuous use of chemical fertilizers can cause damage to the soil and reduce nutrients. According to Jones (1982), inorganic phosphate fertilization on ultisol soils has the main problem: the low effectiveness of P fertilizer, namely 10% to 30%, so that 70% to 90% of P fertilizer remains in the soil and is difficult for plants to absorb. Low fertilizer efficiency causes the amount of inorganic P fertilizer to be applied by farmers to increase so that it has the potential to reduce productivity, so its use needs to be reduced by utilizing biological fertilizers. The use of phosphate solubilizing bacteria can increase the efficiency of P fertilizer.

The use of phosphate solubilizing bacteria (PSB) as an agent to reduce pathogen attacks can increase phosphate availability due to the production of organic acids and phosphatase enzymes; besides, it also functions as a biocontrol agent (Setiawan 2008). Thakuria et al. (2004) stated that phosphate solubilizing bacteria isolated from the rice rhizosphere could increase rice production from 5.4 to 21.6%. Isolation and identification of phosphate solubilizing bacteria from the rhizospheres of rice plants (*O. sativa*) in organic and non-organic rice fields in the Sukoharjo area revealed that Isolates P1 and P13 were 99 % identical to *Acinetobacter* sp. Isolate Q7 had a 96% similarity with the genus *Clavibacter*, and isolate Q8 had a 99% similarity with *P. aeruginosa*.

In conclusion, 24 isolates of phosphate solubilizing bacteria were obtained from organic and non-organic rice fields in the Sukoharjo area. The two lines of *Acinetobacter* sp. with the highest IP were phosphate solubilizing bacteria isolated from organic rice fields. *Pseudomonas aeruginosa* and one isolate with a 96% similarity to *Clavibacter* were the most phosphate solubilizing bacteria isolated from non-organic rice fields with the highest IP.

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