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Composition of Diatom Species in the Internal Organs of White Rats (Rattus norvegicus Berekenhout, 1769) as a Support for Forensic Diagnosis of Drowning Victim Cases

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Abstract – Forensic diagnosis of drowning victims can be done in several ways, one of which is by looking at the diatoms found on the drowning victim's body to see where the drowning victim occurred, the cause of death of the drowning victim, and determining the length of time the drowning victim took place. This study aims to determine the species and characteristics of the diatoms found in the internal organs of white rats (*R. norvegicus*) that were drowned in the Batang Arau River, Padang City and to determine the species of diatoms that can help forensic diagnoses at the drowning victim's crime scene. This research was carried out from October 2022 to January 2023 at the Batang Arau River, Padang City and the Animal Ecology Research Laboratory. The method used in this research is the survey method. In this study, the diatoms found in the internal organs of white rats were 19 species consisting of 147 individuals. Diatom cells are pinnate in shape, the most apex shape variations are *rounded* by 58% and the smallest is rostrate 10%, while the size of diatoms ranges from ±130.50 - ±1132.22 µm. Diatoms that can be used to assist in forensic diagnosis of crime scenes in the Batang Arau estuary are *Eunotia bilunaris* and *Gomphonema pumilum*.

Keywords – Diatoms, Internal Organs, Batang Arau River, Crime Scene, Drowning.

1. Introduction

Diatoms are a group of microalgae which generally have single cells and whose cell walls are enriched in silica (SiO_2). The composition of their cell walls containing silica makes diatoms very resistant to high temperatures and strong acids. The presence of diatoms in various waters can be used as an indication of the location of death cases and the cause of a person's death, whether due to drowning or death and drowning [1]. Drowning is a type of death due to asphyxia which is caused by air covering the respiratory tract to the lungs [2]. When a person drowns or sinks in a body of water, the diatoms in the water will enter the body at the same time as the water is inhaled [3].

In West Sumatra, many drowning victims were found, including in the waters of the Batang Arau River. Several cases of drowning victims found in the waters of the Batang Arau River, Padang City, include the discovery of the bodies of elementary school students, cases of drowning children, cases of finding drowned children, cases of drowning victims. the case of finding the body of a fisherman, the case of finding the body of a man, and the case of finding the body of a teenager [4]. Research on the presence of diatoms to support forensic diagnosis at the scene of the death of a drowning victim has not been carried out much in West Sumatra. Research conducted by Nofrita et al., [5] only obtained diatom types and diatom characteristics in each river in Padang City, and research by Kurniawan, [4] conducted research on diatom colonization and diatom characteristics in diagnosing the duration of

drowning victims and crime scenes using a semen plate. The characteristics of the types of diatoms present in internal organs have not yet been obtained to assist forensic diagnosis of drowning crime scenes. Therefore, it is necessary to conduct research on the composition of diatoms in internal organs as a support for forensic diagnosis of drowning victim cases.

2. Method

This research was conducted using a survey method. Location determination carried out based on community activities and referring to previous locations found a drowned victim. At each location, white rats were drowned (*R. norvegicus*). This immersion was carried out for 24 hours. Drowning white rat (*R. norvegicus*) were carried out on the Batang Arau River, Padang City with coordinates 0 º 48" to 0 º 56" S and 100 º 21 to 100 º 33 E (Figure 1).

2.1 Sampling in the field

sampling was carried out by drowning white mice in the Batang Arau River, Padang City, which consists of 4 stations. White mice that had been drowned for 24 hours were removed from the water in the field and taken to the laboratory for surgery and taking the internal organs of white mice. For collection of diatom samples. Water parameters were measured by measuring the physicochemical parameters of the water at the time of immersion of the white rat.

2.2 Diatom sampling

Diatom sampling in white rat organs This was done by dissecting a white rat to remove the internal organs, then the internal organs of the white rat that had been taken were immediately doused with 500 ml distilled water in a plastic tray and chopped up, after that the water was filtered using a plankton net. The water from the plankton net filter was then put into a 25 ml sample bottle and added with Lugol and 4% formalin as a preservative. The sample bottles are then labeled with station information at each location. The water sample in the sample bottle is then identified and measured using a microscope .

2.3 Identification of diatom samples

Diatom samples obtained from the internal organs of white rats were identified to species level, then individual counts were carried out for each species directly. Observations were made on the morphological form of diatoms, apex shape, size and type of diatoms using identification book by Prescott's [6], Yamaji [7], and Bold and Wyne [8], Masaharu [9] and Referring to www.algaebase.org [10] and www.Marinespecies.org [11]

2.4 Data analysis

The diatoms obtained were then analyzed for diatom species, the characteristics of the general shape, apex shape and size of the diatoms obtained from the identification results were presented in tabular form and compared the diatoms in the internal organs of the white rat's body with data on diatoms in the waters at that location. The same one uses secondary data Kurniawan, [4].

2.5 Ethics

This study was approved by the Andalas University Faculty of Medicine research ethics commission team with reference number 467/UN.16.2/KEP-FK/2023.

3. Results and Discussion

3.1 Composition and characteristics of diatoms in the internal organs of white rats

Based on research conducted by diatoms found in the internal organs of white rats drowned in the Batang Arau River, Padang City, there were 19 species consisting of 147 individuals (Table 1).

Table 1 shows the number of individuals of each species obtained from the internal organs of white mice. The highest number of individuals found in the *Eunotia arcus species* was 22 individuals, while *Cymbella aspera* had the smallest number, namely 1 individual. The large number of individuals of *the E. arcus* species found in the internal organs of white mice is because this species has the ability to survive in all water conditions and *E. arcus* is also widely distributed in various waters with a large number of individuals . According to Alles *et.al.*, [12] that diatom species *E. arcus* has high adaptability and is widespread in waters. *Cymbella aspera* is the species with the smallest number of individuals because this species is only found in few waters. According to Bahls [13], the diatom species *C. aspera* is very rarely found in large numbers in waters.

Figure 2 shows that the location that has the most species and individuals is location 1, namely 11 species consisting of 54 individuals, while the location that has the fewest species is location 4 with five species with 10 individuals. Location 1 is a river estuary where freshwater and seawater mix, so the diatoms found are also a combination of freshwater and marine diatoms, so the number of diatom species and individuals at location 1 is higher than other locations. Kurniawan,[4] also found freshwater and seawater diatoms at one location, namely the Batang Arau River Estuary, Padang City. Locations 3 and 4 are the locations that have the lowest number of individuals and species, this is because at locations 3 and 4 there is rubber factory waste and household waste coming in which causes the location to become polluted, apart from that these two locations have nitrates. and lower phosphate (Table 4) which results in a lack of nutrients for diatom life at that location. This is in accordance with the opinion of Kurniawan,[4] that locations 3 and 4 are locations with polluted waters resulting from rubber factory waste and household waste which results in species being unable to survive in these locations.

The diatoms found in the internal organs of white rats are diatoms from the pinnate group *which* have a general shape of bilateral symmetry and elongation (Figure 3). The dominance of the *pennate* diatom species is due to the fatigue diatom (*Pennate*) found more often in waters, has an elongated, oval cell shape do not have spines, so this pennate diatom *easily* enters the blood circulation and is found in every internal organ of the white rat. This is in accordance with the opinion of Smith *et al.,* [14] who stated that *pennate* diatoms *are* in greater numbers in fresh waters. Nontji, [15] stated that *pennate* diatoms are characterized by bilateral symmetry or oval cell shapes and do not have spines.

The shape of the apex can also be used as a distinguishing character for each diatom species. The variations in the shape of the diatom apex found in the internal organs of white rats are *capitate, rostrate, rounded,* and *subcapitate* (Figure 4) .

The highest percentage of apex shapes of diatom species found in the internal organs of white mice is a *rounded* shape at 58%. Diatoms with a *rounded* apex shape have rounded edges and live in colonies so that if they are inhaled and enter the internal organs of white rats with water there will be a lot of them too. Wehr *et al*., [16] stated that diatoms with a *rounded apex shape* have round valves and have no boundaries to connect with each other so they often live in colonies. The fewest apex forms found in the internal organs of white rats are diatoms with a *rostrate apex form*, namely 10%. There are very few diatoms with a rostral apex shape because this group has a shape with a beaked valve tip, making it impossible for them to live in colonies. Wehr *et al*.,[16] stated that diatoms with a rostrate apex have a valve tip that forms a beak and have limitations for colonizing.

The percentage of diatom apex shapes at each location is different. At each location, the shape of the diatom apex that is mostly found is *rounded*, at location 1 it is 45.45%, location 2 is 42.86%, location 3 is 33.33%, and at location 4 it is 60%, this is due to the number of species Diatoms with *rounded apex shapes* are scattered throughout every body of water. According to Taylor *et al*.,[17] that phytoplankton species with *rounded apex shapes* are often found in waters. Diatoms with a *rounded* apex shape found in the internal organs of white rats are *A. brevipes, C. aspera, E. bilunaris, F. construens, G. pumilum, N. anglica, N. bacillum*, *N.* lanceolata*,* N. *amphibia, P. viridis,* and *R. ghibba.* At location 1, there are 4 apex shapes found in the white rat's internal organs, including rostral capitate, rounded and subcapitate. The large number of diatom apex forms found in the internal organs of white rats is because location 1 is a river estuary which contains lots of nutrients that can support diatom life and the diversity of diatom species at location 1 is due to the influence of fresh water and sea water. According to Nybakken,[18] which states that river estuaries are river estuary areas that have certain characteristics unique, because it is influenced by fresh waters and the sea so that many diatom species are found in river estuaries.

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Apart from the rounded apex shape, at location 2 there are also diatoms with an apex capitate shape which has a high percentage, namely 42.86%. The large number of diatoms with an apex capitate shape at location 2 is because diatom species with an apex shape are often found at location 2, namely *E. arcus, F. capucina* , and *F. crotonensis* . At the location of the 3 diatom apex forms found in the internal organs of white rats, there were only 3, namely capitate 33.33%, rounded 33.33% and subcapitate 33.33%. At location 3, no diatoms with a rostrate apex shape were found, as in other locations, because diatom species with a rostrate apex shape are very rarely found in the waters. The diatom species with a rostrate form is *N. cryptocephala,* according to Cox,[19] who stated that the diatom species *N. cryptocephala* is only found in environments with high nutrient levels. Location 3 is a location with low nutrient levels (Table 3). At the location of the 4 diatom apex shapes found in the internal organs of white mice, namely capitate, rostrate, and rounded. There were no diatoms with a subcapitate apex shape found because diatoms with a subcapitate apex shape mostly live in slightly alkaline waters, diatoms with a subcapitate apex shape are in the genus *Synedra*. According to Bere,[20] who stated that *Synedra* can be found in epilithic and epiphytic biofilms that are slightly alkaline, oligosaprobic, and eutrophic. At location 4 there are waters with an acidic pH of 6.3 (Table 4) so that diatom species from the genus Synedra are not found at location 4.

The internal organs of white rats vary in size (Table 3). According to Xue et *al*.,[21] diatoms in waters have varying sizes, the size of diatoms generally ranges from 5 µm–2 mm.

It can be seen in Table 3, the highest average size of diatom species found in internal organs is at location 1 while the lowest is at location 3. For example, the species *F. capucina* has different average sizes at each location. The average size of the diatom species *F. capucina* was highest at location 1, with an average size of 1,353.82 µm and the lowest was at location 3, namely with an average size of 444.86 µm. Location 1 is an estuary area where in the estuary there is a lot of accumulated waste from the upstream river which can later cause high levels of nitrate and phosphate which can be beneficial for the growth of diatoms. This is in accordance with the opinion of Boney[22], who stated that the abundance of diatoms can be influenced by nutrient elements such as nitrate and phosphate . According to Mackentum (1969) *in* Yuliana and Tamrin[23], optimal growth of phytoplankton requires a nitrate content in the range of 0.028-3.5 mg/l. In the Batang Arau River, nitrate levels range between 0.553 - 0.731 mg/l and phosphate ranges from 0.020 - 0.057 mg/l. At location 1 is the location that has the highest levels of nitrate and phosphate, namely 0.731 mg/l and 0.057 mg/l, while at location 3 is the location that has the lowest levels of nitrate and phosphate, namely 0.553 mg/l and 0.020 mg/l (Table 4). According to Soedibjo[24] the optimum phosphate value for diatom growth is 0.018 - 27.8 mg/l. The high number of species and individuals in location 1 is also thought to be due to the large number of species from the genus *Navicula* found in the internal organs of white rats at location 1. According to Isnanstyo and Kurniastuty[25], the genus N*avicula* can grow optimally in waters with a pH range of 6－8 and a temperature range of 22 °－30 °C. This is in accordance with the results obtained in research where the pH obtained was 6 and the temperature obtained was 28° C (Table 4) which resulted in optimal life of diatoms from this genus.

3.2 Diatom species in the internal organs of white rats found at each location

Based on research that has been carried out, the species found in each location have different diatom species . In Table 8 you can see the diatom species in the internal organs of white rats found at each research location, namely in the Batang Arau River, Padang City. The differences in diatom species at each location will indicate the presence of unique diatoms at each location, because diatom species that are only found at certain locations are typical diatom species. This is in accordance with the opinion of Kurniawan[4], who states that each location has unique diatoms that can only be found in each location. Diatoms that are unique to each location can also be used to assist forensic diagnosis to determine the scene of a drowning victim, namely by looking at the diatoms found in internal organs and those in the water (Table 5).

Based on Table 5, there is a comparison of diatom species in the internal organs of white rats with previous research conducted by Kurniawan[4], which shows that there are differences in the species in internal organs and those in water, this is due to differences in the ability of diatom species to can enter the internal organs of white mice and differences in adaptability to the environment. Apart from that, we can see that diatoms that can support forensic diagnosis at the scene of a drowning victim are the species *Eunotia bilunaris* and *Gomphonema pumilum*. According to Horton *et al*.,[26] and Punia[1], diatom species typical of a body of

water can be used to help reveal the location of death or crime scene (TKP) in cases of death caused by drowning. The species *E. bilunaris* and *G. pumilum* are diatoms that are typical for location 1, because these species are only found at location 1, both in internal organs and in the water. At locations 2, 3, and 4, no diatom species were found that could help in determining the scene of the drowning victim because no diatom species were found in the internal organs of white rats and they were also found in the water. Rohn and Frade[27], stated that the discovery of diatoms in the organs of drowning victims and in the water can help identify the location of death or the crime scene.

4. Conclusion

The conclusions obtained based on the research conducted are The diatoms found in the internal organs of white mice consisted of 19 species and 147 individuals. Diatom cells are pinnate in shape, the largest variation in apex shape is *rounded* at 58% and the smallest is *rostrate* at 10%, while the variation in diatom size ranges from ± 130.50 -±1,132.22 µm. Diatoms that can be used to assist forensic diagnosis of crime scenes in Batang Arau river estuary are *Eunotia bilunaris* and *Gomphonema pumilum.*

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Table 1

Table 1. Composition of diatoms found in the internal organs of white rats drowned in the Batang Arau River, Padang City

No	Species	Amount
1	*Achnanthes brevipes*	16
2	*Cymbella aspera*	1
3	*Cymbella tumida*	8
4	*Cymbella ventricosa*	6
5	*Eunotia arcus*	22
6	*Eunotia bilunaris*	4
7	*Fragilaria capucina*	14
8	*Fragilaria construens*	7
9	*Fragilaria crotonensis*	2
10	*Synedra acus*	8
11	*Synedra ulna*	11
12	*Gomphonema pumilum*	3
13	*Navicula anglica*	9
14	*Navicula bacillum*	11
15	*Navicula cryptocephala*	6
16	*Navicula lanceolata*	3
17	*Nitzschia amphibia*	6
18	*Pinnularia viridis*	8
19	*Rhopalodia gibba*	2
	Total	147

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Table 2

Table 2. Percentage of diatom apex forms in the internal organs of white rats at each location

Apex shape	Percentage (%)			
	Location 1	Location 2	Location 3	Location 4
Capitate	18.18	42.86	33.33	20
Rostrate	18.18	0	0	20
Rounded	45.45	42.86	33.33	60
Subcapitate	18.18	14.29	33.33	0

Note: Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung.

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Table 3

Table 3. Variations in the average size of diatoms found in the internal organs of white rats at each location

No	Species	Average diatom size (µm)			
		Location			
		1	2	3	4
1	*Achnanthes brevipes*	-	395.71	-	-
2	*Cymbella aspera*	-	-	-	210.61
3	*Cymbella tumida*	344.11	-	-	383.65
4	*Cymbella ventricosa*	330.53	340.94	-	-
5	*Eunotia arcus*	462.74	306.86	260.59	-
6	*Eunotia bilunaris*	1,026.12	-	-	-
7	*Fragilaria capucina*	1,353.82	1,052.19	444.86	832.09
8	*Fragilaria construens*	219.20	-	-	-
9	*Fragilaria crotonensis*	-	245.89	-	-
10	*Synedra acus*	-	-	1,132.22	-
11	*Synedra ulna*	1,148.17	-	648.97	-
12	*Gomphonema pumilum*	234.02	-	-	-
13	*Navicula anglica*	193.62	-	161.74	-
14	*Navicula bacillum*	120.09	-	-	140.91
15	*Navicula cryptocephala*	239.02	-	-	-
16	*Navicula lanceolata*	-	249.40	-	-
17	*Nitzschia amphibia*	-	-	237.38	-
18	*Pinnularia viridis*	-	656.50	-	-
19	*Rhopalodia gibba*	-	-	-	477.19

Note: (-): not found, Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung.

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Table 4

Table 4 . Physico-chemical factors of Batang Arau River waters, Padang City

No	Physico-chemical facts	Location 1	Location 2	Location 3	Location 4
1	Water temperature (^{o}C)	28	26	26	25
2	Current speed (cm/s)	2.93	1.39	6.01	6.14
3	pH	6	6.58	6.5	6.3
4	Dissolved oxygen (ppm)	2.6	2.52	4.8	4.2
5	BOD (ppm)	0.3	0.56	0.57	0.78
6	Free carbon dioxide (ppm)	0.26	0.34	0.23	0.38
7	Salinity ($^{o}/_{oo}$)	7	0	0	0
8	Nitrite (mg/l)	0.136	0.046	0.012	0.005
9	Nitrate (mg/l)	0.731	0.636	0.553	0.602
10	Phosphate (mg/l)	0.057	0.037	0.020	0.044
11	Silicate (mg/l)	3.56	3.92	4.91	3.67

Note: Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung.

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Table 5

Table 5. Diatom species on internal organs and the waters of the Batang Arau River

No	Species	Internal organs				Waters			
		Location				Location			
		1	2	3	4	1	2	3	4
1	*Achnanthes brevipes*	-	√	-	-	√	√	-	-
2	*Cymbella aspera*	-	-	-	√	√	√	√	√
3	*Cymbella tumida*	√	-	-	√	√	√	√	√
4	*Cymbella ventricosa*	√	√	-	-	-	√	√	√
5	*Eunotia arcus*	√	√	√	-	√	√	√	√
6	*Eunotia bilunaris*	√	-	-	-	√	-	-	-
7	*Fragilaria capucina*	√	√	√	√	√	√	√	√
8	*Fragilaria construens*	√	-	-	-	√	√	√	√
9	*Fragilaria crotonensis*	-	√	-	-	√	√	√	√
10	*Synedra acus*	-	-	√	-	-	√	√	√
11	*Synedra ulna*	√	-	√	-	√	√	√	√
12	*Gomphonema pumilum*	√	-	-	-	√	-	-	-
13	*Navicula anglica*	√	-	√	-	-	-	-	-
14	*Navicula bacillum*	√	-	-	√	√	√	√	√
15	*Navicula cryptocephala*	√	-	-	-	√	√	√	√
16	*Navicula lanceolata*	-	√	-	-	√	√	√	√
17	*Nitzschia amphibia*	-	-	√	-	√	√	√	√
18	*Pinnularia viridis*	-	√	-	-	√	√	√	-
19	*Rhopalodia gibba*	-	-	-	√	-	-	√	√

Note: (√) : found, (-) : not found, Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung

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Figure 1

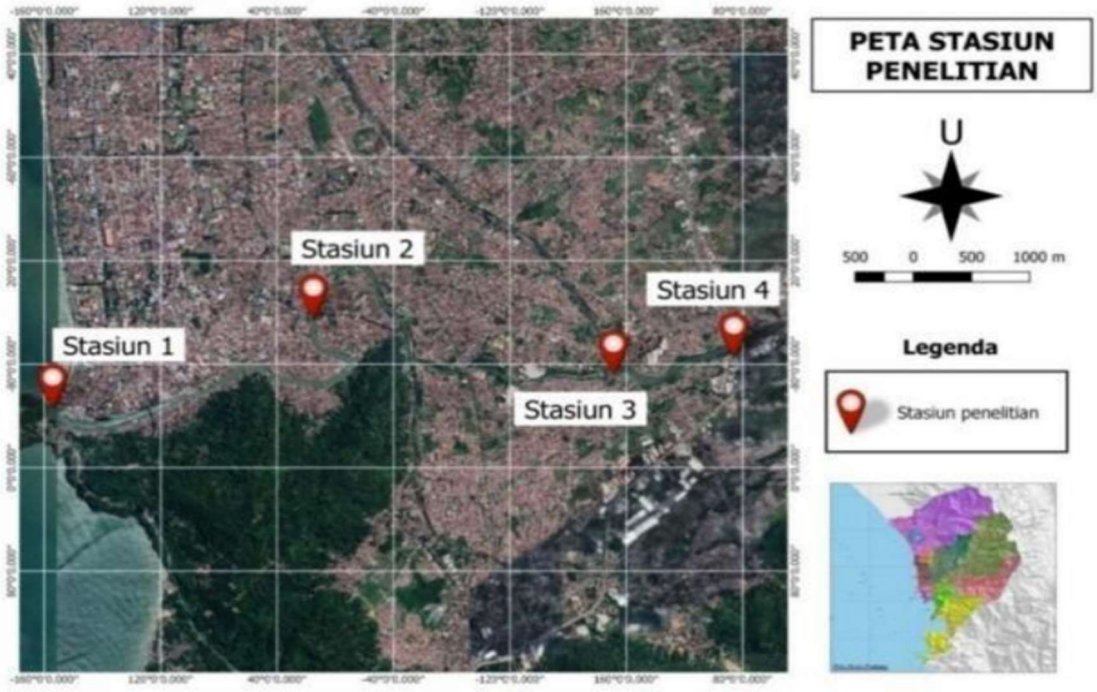

Figure 1. Map of Diatom Sampling Locations (Source: QGIS accessed 15 July 2023)

Note: Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung.

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Figure 2

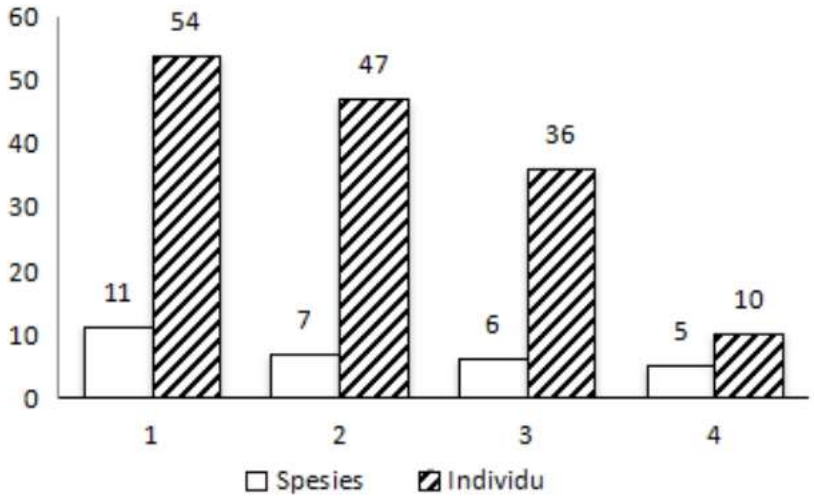

Figure 2 . Total number of diatom species and individuals in the internal organs of white rats at each location

Note : Location 1: Muaro Padang Beach, Location 2: Seberang Padang, Location 3: Tj. Saba Pitameh, Location 4: Parak Laweh Pulau Air Nan XX, Lubuk Begalung.

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Figure 3

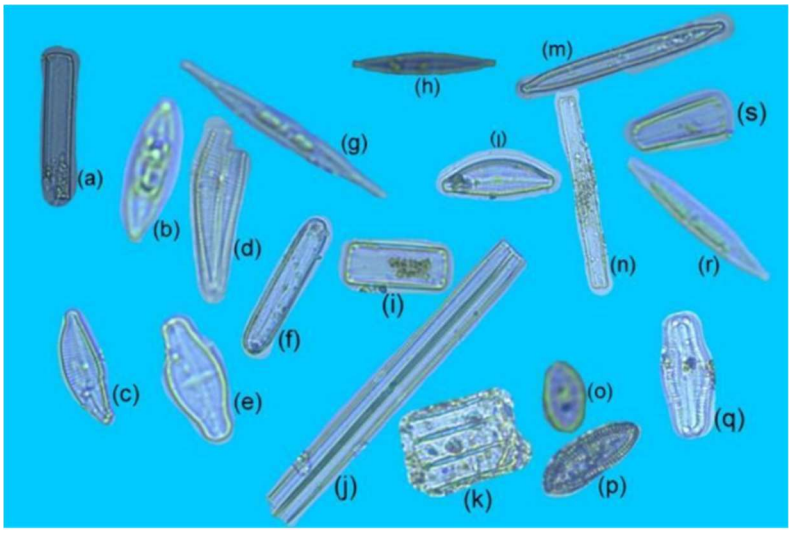

Figure 3 . Variations in the shape of diatom cells found in the internal organs of white rats (Personal documentation)

Note: (a) *E. bilunaris*, (b) *N. lanceolata*, (c) *C. ventricosa*, (d) *C. aspera*, (e) *N. anglica*, (f) *P. viridis*, (g) *F. crotonensis*, (h) *N. amphibia*, (i) *E. arcus*, (j) *S. acus*, (k) *F. construens*, (l) *C. tumida*, (m) *S. ulna*, (n) *F. capucina*, (o) *N. bacillum*, (p) *A. brevipes*, (q) *R. gibba*, (r) *N. cryptocephala*, (s) *G. pumilum*

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Figure 4

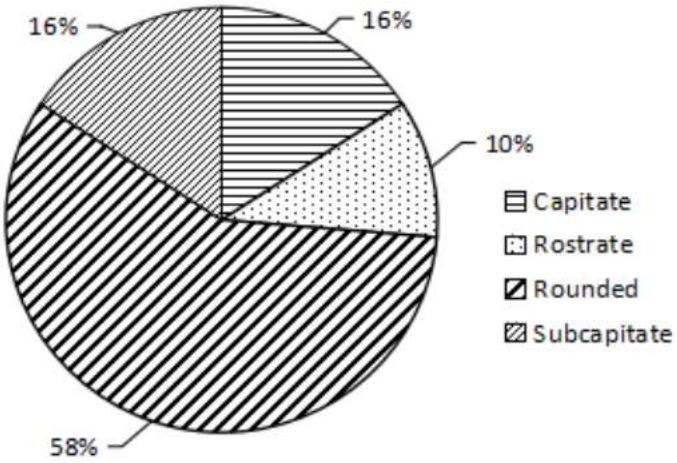

Figure 4. Percentage of diatom apex forms in the internal organs of white rat.