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Original Research Article

Decomposition Mediated Alteration in Scale Structure Assessed Through SEM in Teleosts of River Godavari, Maharashtra, India

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Abstract: The ultra-structure study of fish body scales using SEM is an important tool now a days found useful in fish Systematics. Almost all types of scales are well known for their morphology and can be distinguished however there are specific patterns of arrangements and types of ultra-structures found on scale surfaces like circulli, radii, ctinii, tubercles, spines, etc. These structures are species-specific hence if the only scale is available as a sample from the habitat then the fish may be identified based on scale structure. To such an extent the taxonomical records are available and that need to be updated. But when scales lost from the fish body and become very old due to changes in surface ultra-structures and scale as a whole may cause difficulty in the identification. The soil, sand, organic matter, dissolved solids in the water, and pollutants affect considerably to cause changes in scale structure. In the present investigation commonly occurring four teleosts of food value from the river Godavari at Nanded, Maharashtra State of India were selected as model sample species. The body scales were subjected to long-term exposure with soil and sand collected from the habitat itself in the well-maintained laboratory conditions by maintaining 80% soil humidity and 25 to 30 °C temprature and changes in scale shape and morphology of ultra-structures were examined under the SEM. The SEM of normal scales were used as a control. The soil and sand were found to cause considerable changes in the scale associated structures. The data will be useful in toxicological, environmental, and Ichthyological investigations.
Key words: Godavari River, Fish Scales Decomposition, SEM

Introduction

Fishes are one of the most primitive groups of vertebrates, with a fossil record dating back more than 500 million years in the Devonian period. The study of fishes is essential in various ways for the wellbeing of human race on this globe. They are important as food, recreation, and pets too. Fishes are an important part of aquatic food webs in both freshwater and marine ecosystems (Sumayya and Chavan, 2017). Taxonomic identification of fish is essential to conserve them and to determine their role in the aquatic ecosystem (Sumayya *et. al.*, 2016). Fish scales have remarkable numerous microstructures and scales act as protective exoskeletal parts on fish body to prevent injuries, pathogenic infection to the skin. The scales are modified as armors or stings in some fish species. They are of various forms. The types of scales as well as the number of rows and total number of scales along lateral line are some important characteristics considered for species identification (Humera *et. al.*, 2015). The morphological diversity and functions of scales in fish always attracted many researchers. Surprisingly a very few studies have contributed significantly to the knowledge about fish scale morphology as it related to the taxonomy of bony fishes. There are several reports on the study of fish scale. Agassiz (1833) was the first who divided the fishes into four groups based on the structure of scales. Later on Cockerel (1913) described scale characters such as different shapes of scales, length, and width, the position of focus and radii and circulli, etc. Hubbs (1921) observed the characters of ctenii in *Mugilid* species and found variations that gives a reliable key for identification to species level. Lagler (1947) noted the morphological characters of scales and its use for diagnostic purposes of major taxonomic levels upto families. Pillay (1951) discussed the affinities of *mugilid* scales and their importance in evolution. Van Oosten (1957) recognized the importance of scales in fish taxonomy. Mc Cully (1961) studied the comparative anatomy of *serranid* fish scales and its SEM studies for the systematics. (De Lamates and Courtenay, 1974; Hughes, 1981), Jawad (2005) identified the most useful scale characters and their importance in the systematics of *tripterygiidae*. Brraich and Jangu (2012) investigated on importance of scale studies as pollution markers, in Harike wetland, Punjab, India. The present study deals with the changes in microstructures of scales due to decomposition that to what extent the microstructures like ctenii, focus, radii, tubercles, marginal area of scales, changed from normal due to impact of polluted water and soil pollutants. The main idea was first to determine the structure of a normal scale by using SEM then the same scale was subjected for exposure to normal soil and sand from the river and the changes in scale microstructure were observed as control and changes in scales to controlled pollution conditions.

The present study is on some important preliminary structural changes in scales due to pollution. The study will be having an application in scale structure-based fish taxonomy of fishes. The scales in normal or control and experimental were assessed first time using SEM in the selected teleost species from river Godavari. This study will be also useful to determine the role of fish in aquatic food chain by gut contain analysis.

Materials and methods

Four fish species were selected i.e. *Catla catla* (Hamilton, 1822), *Etroplus surantensis* (Bloch, 1790), *Notopterous kapirat* (Pallas, 1769), *Macrognathus pancalus* (Hamilton, 1822). the fish species selected in this study are common food fishes as model species and due to variation in their scale morphology. The fish samples were collected from the local fish market named Itwara bazaar in Nanded city of Maharashtra where the major catch from river Godavari flowing from Nanded city. The scales were removed from the body of these fishes brought to laboratory, washed by normal tap water then by Distilled water. Eight clay pots of 2kg capacity were selected. Four of the pots were filled with sand and the other four with soil. Soil was a blend of mud, organic particles, and the natural debris of sand. Soil, silt, and water are used as base materials for the decomposition process. It was collected from the Godavari river at Nanded city, Maharashtra. The fish scales were subjected at 10 cm depth below the soil in the experimental pots (1lit capacity). The pots were filled with soil, silt, sand, and water. The water from Godavari River was used regularly to maintain wetness temperature, turbidity, in the decomposition process. Soil analysis tests performed after 12 months using soil testing kit: ko54 soil testing kit, (HiMedia Laboratories Pvt, Ltd). After following the procedure given in the protocol guideline and soil test were carried for PH (acidity or alkalinity) organic carbon, phosphate, potassium, ammonical nitrogen, and nitrate nitrogen. The scales were weighed in grams before subjecting to the decomposition process. After twelve months the scales from experimental set were removed from the decomposition process and again weighed. Due to the decompositions process, there was some loss in the weight of the scales (Table.1).

Preparation of scales observation under Scanning Electron Microscope (SEM)

The scales were removed carefully from the soil after 12 months to observe the deterioration changes in the scales, SEM micro-photo were used to observe the decomposition mediated changes in the morphology of scale proper and various microstructures on it. For SEM observation excess soil was

Table 1. Change in weight before and after decomposition of scales in four teleost species.

Sr. No.	Name of Fish	Weight of scales in (gm)		
		Before decomposition	After decomposition	
			In soil	In sand
1.	*Catla catla*	5.0	±1.65	±1.75
2.	*Etroplus surantensis*	1.0	±0.18	±0.20
3.	*Notopterus kapirat*	1.0	±0.50	±0.30
4.	*Macrognathus pancalus*	1.45	±0.10	±0.12

removed from the scales and washed with distilled water and dehydrated with 70% ethanol only. The scales were not processed in 100% ethanol or acetone because it causes a curling effect on the scale margin. Scales were cleaned and dried for 2 min and for SEM they were mounted on metallic stub (aluminium blocks) using carbon double adhesive tape in the manner that the dorsal surface will remain upward and the ventral surface stick to the tape and sputtered with a layer of gold nano-particles in a gold coating unit (thikness100A, JEOL JSM-6100) then kept under vaccum using electron gun at an accelerating voltage of 20.0 kV of SEM. Then SEM images of scales were clicked at different magnifications by the digital camera of the SEM, which was connected to a monitor of a computer. The SEM photographs of normal scales were used as a control to compare the changes in surface ultra-structures.

Results

SEM microphotographs show morphological deformities in the scales (Fig. B, D, F, and H).

The figures from A-1 to H-56 (Table.2) shows normal, soil exposed and sand exposed changes in scales. The decomposition induced changes in the scales of selected fish species in the laboratory condition revealed through SEM are explained as below-

1. ***Catla catla:*** In the species by comparing normal to decomposed scales it revealed that the dorsal surface of normal scale under the SEM (Fig. A -1 to 4) of *Catla catla* scale was having a flat surface. The radii and circulli were easily distinguished, many and very close to each other. There was a considerable thickness of scale as a whole due to the presence of a closely arranged large number of circular and radii. The decomposed scale of *Catla catla* observed under SEM showed blur and faint circulli and radii. (Fig. B-5). When further closely observed it revealed broken and damaged circulli and radii. (Fig. B- 5 to 8). scales of *Catla catla* which was decomposed in the sand was observed under SEM, it showed similar changes as found in soil decomposition. (Fig. B - 9 to 12).

2. ***Etroplus surantensis***

The normal scale of *Etroplus surantensis* observed under SEM showed a general morphological view of scale (Fig. C-13). Fig. C-14 & 15 showed clear circuli and radii with a focus in the center. The C- 16 & 17 showed tubercles were found on the posterior side of the scale surface. (Fig. C- 18 to 22) showed the arrangement of small denticles or tongue-like lepidonts on circuli. Lepidonts are prominent tiny, slender, and sharply pointed, widely and closely spaced (Fig. C- 18 & 19). The Lepidonts are firmly attached to the circulii in the deep sockets (Fig. C- 20 & 21). Decomposed scales of *Etroplus surantensis* in soil when observed through SEM revealed uprooting and sloughing off-row from their original position (Fig. D- 23 & 24), no attachments of the circulli (Fig. D- 25-27). The (Fig. D- 28 to 32) showed the decomposed scale in the sand. Damaged focus, broken marginal surface, and cracks in radii were found.

3. ***Notopterus kapirat*** : The scales of *Notopterus kapirat* observed under SEM revealed an overall dorsal view with clear morphological structures (Fig. E- 33). The scale has a very prominent large rounded shape and having thread-like structures (Fig. E – 34 to 36) the circulli exhibit a specific pattern (Fig. E- 37 & 38).

After the decomposition of *Notopterus kapirat* scale observed under SEM revealed various damages in its morphology. The scales that put under soil decomposition experimental setup show severe damage of focus. Focus almost

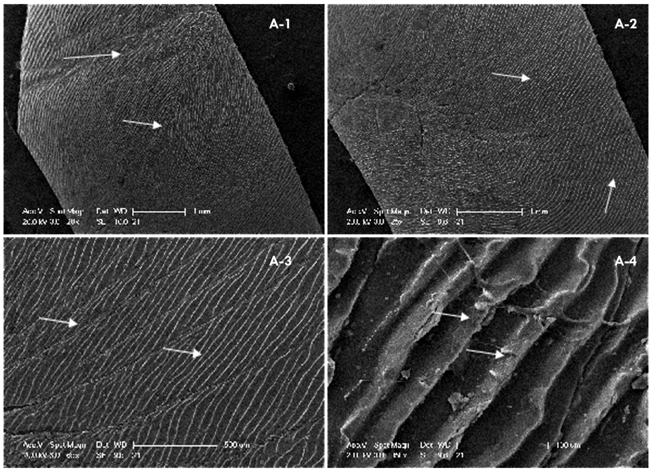

Fig. A. SEM of Normal / Control scale of *Catla catla* (Fig A. 1 to 4). The circuli and radii on scale surface.

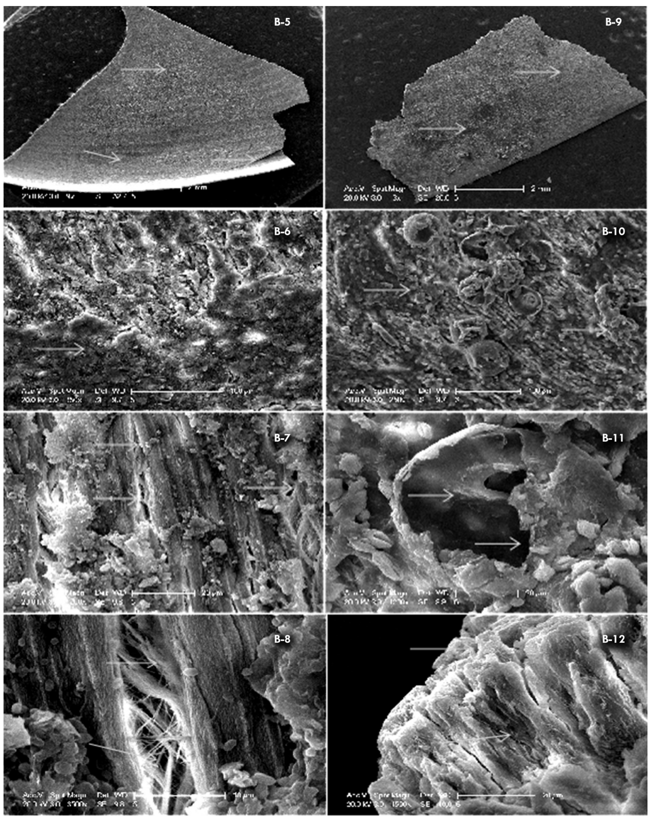

Fig. B. SEM of a part of the decomposed scale of *Catla catla* Exposed to Soil (Fig B. 5 to 8) and exposed to Sand (Fig B. 9 to 12). The alterations in scale surface which lost its morphological characters a deeply decomposed ruptured surface and thickness of the surface.

lost its thread-like structure (Fig. F- 39 & 40). showed broken circulli and radii were found as in (Fig. F- 41 & 42). In sand decomposition conditions the SEM images revealed various

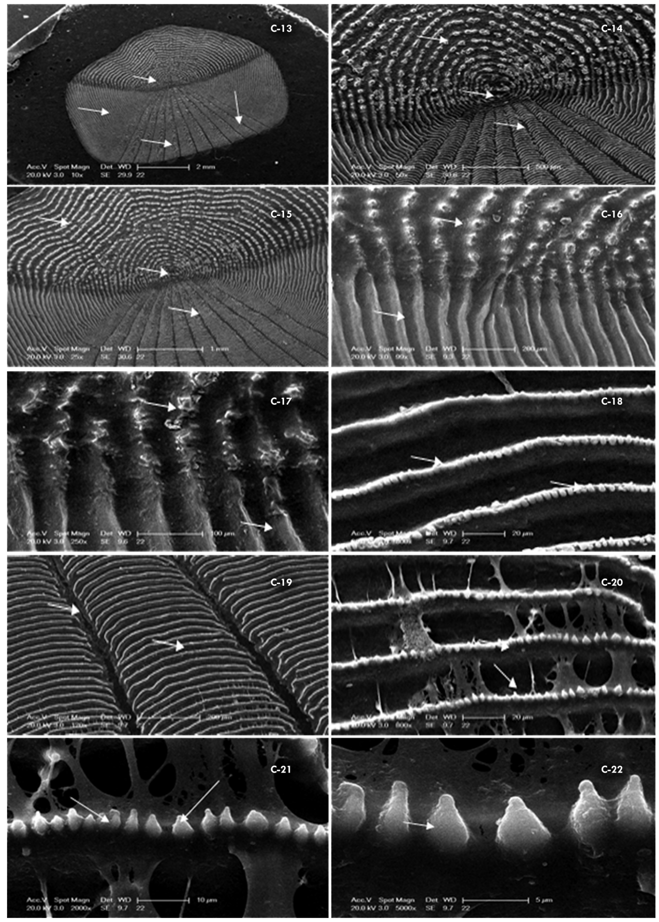

Fig. C. SEM of the Normal / Control scale of *Etroplus surantensis* (Fig C.13 to 22) Scale surface showing focus, circuli, radii, lepidonts, Tubercles. Tubercles (Fig C.13 to17) are small round projections on the surface of scale and it covered the partially scale's surface. Lepidonts (Fig C.18 to 22) are prominent tiny or small, slender and sharply pointed, widely and closely spaced and lepidonts are firmly attached to the crest of the circuli in the deep sockets.

deformities like cracks in radii (Fig. F- 43), damaged surface (Fig. F- 44 & 45) and broken margin of the scale (Fig. F- 46).

4. *Macrognathus pancalus*:

The normal scale of *Macrognathus pancalus* observed under SEM shows the shape of scale is rectangular and flat with a wavy margin. From the focus, radii arise and run towards the anterior margin of the scale. Circuli are many in number, clear and thick. (Fig. G- 47 to 50). The soil decomposed scale of *M. pancalus* in soil shows a completely damaged scale surface (Fig. H- 51). Damages in focus and cracked circulii and radii (Fig. H- 52 & 53). The sand decomposed scale of *M. pancalus* by SEM revealed the same result as found under

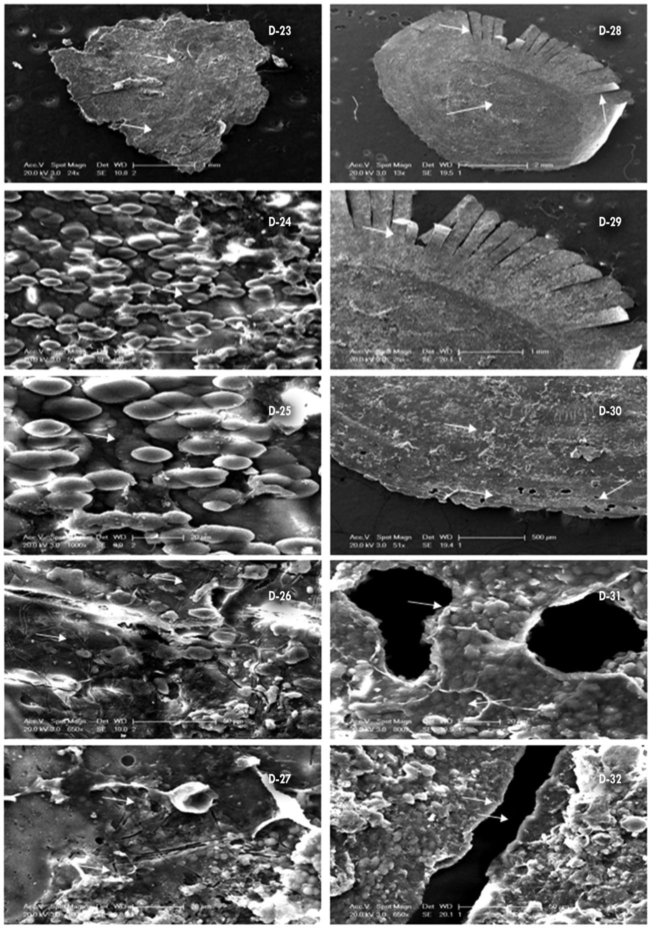

Fig. D. SEM of Decomposed scales of *Etroplus surantensis*, Soil exposed (Fig. D. 23 to 27) and sand exposed (Fig. D. 28 to 32) revealed uprooting and sloughing off Tubercles and a row of lepidonts from their original position, no attachments of the lepidonts on the crest of the circuli. Damaged focus, broken marginal surface, crakes in radii.

the soil, the scale morphology was damaged (Fig. H-54) and the circuli, radii were separated (Fig. H- 55 & 56).

Discussion

Robert et al., (2010), reported that some of the characteristic anatomical features of early vertebrate fossils have been badly affected by decomposition, and in some cases may have rotted away completely. Knowing how decomposition affected the fossils means reconstructions of our earliest ancestors will be more scientifically accurate. The present study demonstrates that the morphology and ultra-structures on scale surface of fishes change due to decomposition and damages seen in the scale structure due to the effect of polluted soil and sand

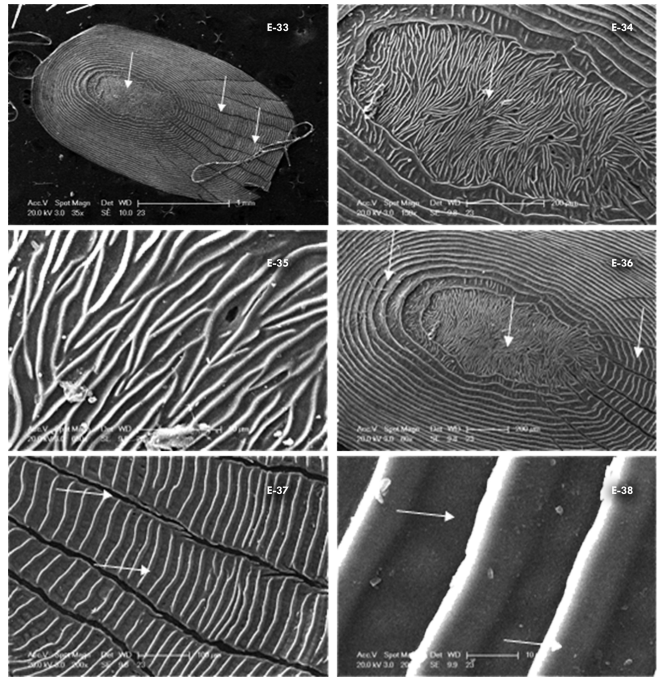

Fig. E. SEM of *Notopterus kapirat*, Normal / Control (Fig. E. 33 to 38) surface showing focus has a threads like structer, many oval shape circuli cover the complete surface of scale and radii also present on the scale surface.

from the natural sources. On exposure of soil and sand to four species of teleost fish *Catla catla* (Hamilton,1822), *Etroplus surantensis* (Bloch,1790*), Notopterous kapirat* (Pallas,1769), *Macrognathus pancalus* (Hamilton,1822). The morphology of decomposed scales after twelve months was compared with control scales. The deformities in the decomposition exposed scales were observed. The ultrastructure of the scales was changed when observed through SEM. The deformities were mainly found in the margins of the scales become irregular similarly, the destruction was initiated in the central part of the focus. The area in scales with developing focus were disturbed and the alternative lines of radii were not clear and were overlapped. The annuli of the scales were disappeared. The annular surface was rough.A different type of particular pattern due to decomposition was developed in every scale (Fig. B, D, F, H).

Rajinder and Mandeep (2015) reported in SEM studies that, there are changes in the dorsal surface of scales of *Catla catla.* Deformities in the focus region of each scale were found. While each normal scale has a distinct focus, radaii, and concentric rings called circuli and other structures

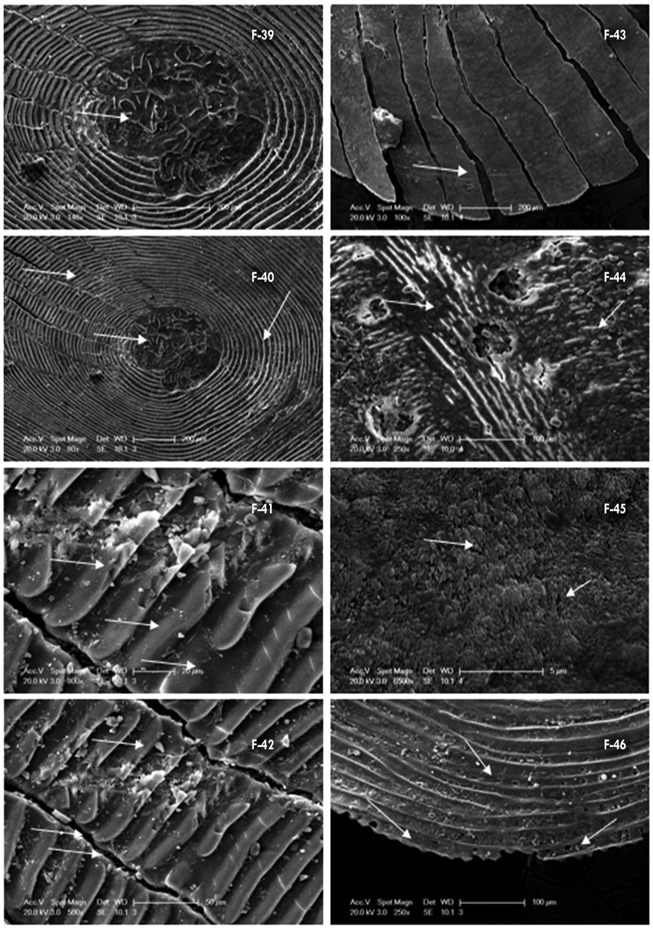

Fig. F. SEM of decomposed scale of *Notopterus kapirat*, Soil exposed (Fig. F. 39 to 42) and Sand exposed (Fig. F. 43 to 46) revealed threads like structures of focus surface almost disappeared, crakes on the surface, distorted pattern in radii and circulii, destroyed margin.

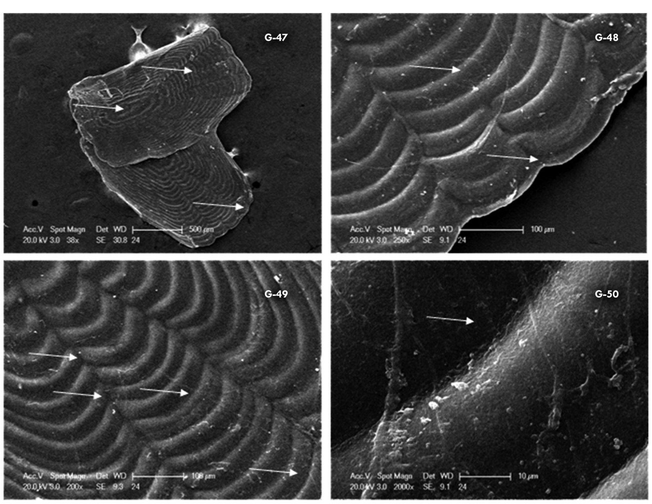

Fig. G. SEM of Normal / Control Scale of *Macrognathus pancalus* (Fig. G. 47 to 50) Showing clearly focus, wavy margin, radii, and many circuli on the surface of scales.

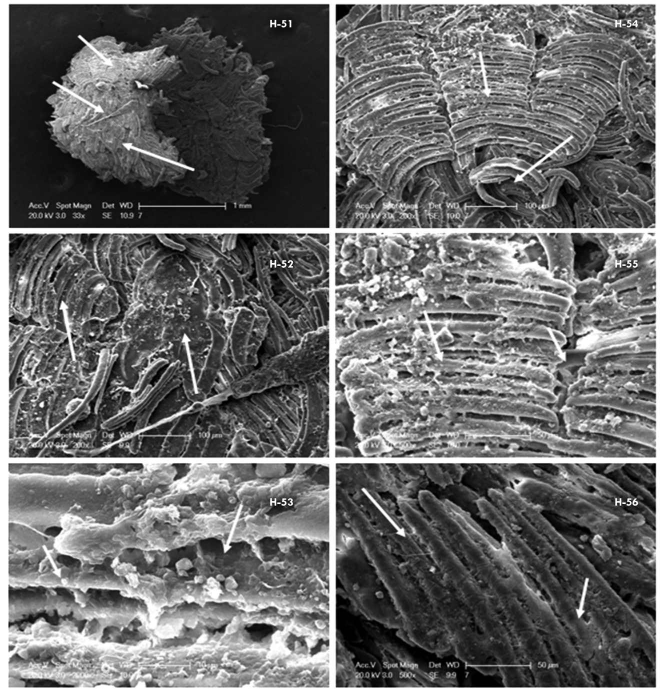

Fig. H. SEM of decomposed scales of *Macrognathus pancalus* Soil exposed (Fig. H 51 to 53) and sand exposed (Fig. H 54 to 56) revealed disintegration and damages of circuli, radii and complete damage of focus and destroyed margins of whole scales.

around it. The focus region of the scales observed in decomposed scales showed deformities ranging from broken lines of radii, and indefinable focus, circulli, and radii around the focus started to disintegrate and break up into small parts leading to many damaged and broken circuli. That also created a space around the focus in comparison to normal scales. Furthermore, exposure of scales to decomposition state revealed pronounced damage in the form of uprooted lepidont in *Etroplus* and sloughing off of whole rows of lepidonts on the scales surface. On the scale surface of *Etroplus,* the intensity of lepidontal damage was more severe and almost all lepidonts disappeared (Fig. C). "The Lepidonts are small denticles or tooth-like micro structures firmly anchored to circuli of the rostral and lateral field that only become visible at high magnification of >2000 X (Zainab Gholami, 2013). In *Macrognathus pancalus,* the scales are of the entire calcareous material become disorganized along the circulli and radii. In *Etroplus* the scale damage was extended to the posterior region of scales as found in the form of disintegrated tubercles.

Although alteration in-focus region, radii, circulli, chromatophores, lepidonts and tubercles, margins of scales, denticle parts were noted in the morphology of fish scales. The intensity of damage was varied from species to species. The SEM photographs of normal scales clearly show various characters but after decomposition alteration in scale morphology was found. There were disorganization and loss of identity upon exposure to soil and sand for a long period. Khanna *et al.* (2007) observed that anthropogenic activities along with increased levels of total solids, turbidity, BOD, COD, and some metals may be associated with severe irreversible damages to the circulli and lepidonts resulting in loosening of scales on the fish body. Brraich and Jangu (2012) reported structural damage in the form of lesions and cracks in the circulli, uprooting of lepidonts and sloughing off lepidonts from their point of attachment to circulli in scales of *L. rohita* collected from Harike wetland in Punjab, India. The study was further supported by Kaur and Dua (2012), on examination of *C. punctatus* exposed to wastewater and found alterations such as loosening of scales due to uprooting and sloughing off of lepidonts and destructions at the circuli and radii levels. Pala *et al.* (2013) noted partially or completely damaged lepidont, dislocation or even a complete row of lepidonts and loss of symmetry of circulli, as well as lesions on the surface of lateral circuli, in scales of *Channa gachua*, inhabiting polluted sites of a hill stream, Umkhrah of Meghalaya, India. It was an indication of clear effect reflection of pollutants on scale microstructures.

Conclusion

It was found that scales in the teleosts were affected due to the action of the soil and sand. The ultra-structures examined through SEM of the scales were partially damaged. In the current study, the period for the decomposition of scales is a long process almost it was for one year. It takes years as bones that take much time for their decomposition. In the present study, it was observed that the exposure of scales to the soil and sand based decomposition model causes significant changes in the ultra-structure. It was finally concluded that scales at an ultra-structure level disintegrate badly due to exposure to pollutants, soil, and sand. In the study to compare with normal, the SEM is the most essential tool. The study may have considerable application is paleotaxonomy of fishes and morphotaxonomy based on scale structure.

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