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ATP-Bioluminescence Assay for Real Time Surface Hygiene Monitoring in Fish Value Chain

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Abstract

ATP-bioluminescence (ATP-B) assay was used to evaluate organic debris load, which in turn indicates the cleanliness of fish contact surfaces (FCS) in fish value chain, where the last point is ready to eat (RTE) fish. Contaminated FCS can harbour lot of microorganisms including pathogens and specific spoilage organisms. From the stipulated four critical points in the value chain *viz.,* harvesting unit (coracle), auction cum assortment area (AAA), pre-processing area (PPA), cooking and marketing area (CMA). Specific FCS were selected for real time surface hygiene monitoring using ATP bioluminescence assay both following normal cleaning process (NCP) and prescribed Sanitation Standard Operating Procedures (SSOP). The present study has significance in fish industry for monitoring hygiene and establishing high risk zones in value chain, as it gives real time information about hygiene once critical limits were located after a justification period. Visual assessment of cleanliness is unreliable in monitoring surface hygiene in fish value chain, and ATP-B testing can aid in the design, execution and substantiation of effectual surface cleaning. For successful monitoring and validation of surface cleaning, a scheme that integrates visual judgment and ATP-B testing, with periodical conventional microbial swab testing is anticipated. The principal component analysis illustratively divided the ATP bioluminescence values of FCS following normal cleaning process (NCP) and prescribed Sanitation Standard Operating Procedures (SSOP).

Keywords: Adenosine triphosphate-bioluminescence, hygiene monitoring, principle component analysis, safety and quality, value chain

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Introduction

The Value Chain approach, which provides a systematic outline for crafting organized and wide-spread strategies to guide the systematic progress, can be a constructive means in the management of fisheries and aquaculture. (Kaplinsky & Morris, 2001) opined that the value chain includes complete array of actions, necessary to bring a product or service from commencement through the different stages of production, till the delivery to end user. Nowadays, fish and fish products have become the most traded food items at international level. Luu (2016) reported that fish is susceptible to contamination throughout the distribution chain. Along the distribution chain, from capture to distribution, the fish comes in contact with surfaces of handling utensils, apparatus, personnel, ice and washing water within the production area. All through this contact, contamination by microorganisms may crop up through the water, personnel and insufficient clean-up procedures. Effective controls and inspections in the fisheries distribution chain are of high significance in terms of food safety as well as consumer safety.

Safety and quality are of supreme importance to the fishery industry as fish and fishery produce are extremely perishable. The safety and quality of seafood depends on its state at harvest, and the way in which it is treated and stored before reaching the end user. This includes contamination of water body, hygiene (on board the fishing vessel, at the landing centre, during transport, storage and marketing), personal hygiene and habits of workers and cleanliness of the environment. Processing of fish can enhance contamination due to direct contact of produce with contaminated surfaces, equipments, water or personnel.

Microorganisms are the major cause of spoilage in most seafood-based value-added products as well as byproducts. Fishes are more perishable than other protein foods and thus more prone to bacterial contamination. Improper sanitary conditions, starting from harvest to the end user, and the incidence of a wide range of food borne outbreaks produce vulnerability in seafood safety. Hence, it is increasingly important to examine and verify the seafood safety risks along the entire seafood distribution chain. Many bacterial species are native component of seafood, but they can also be found on the food contact surfaces, where they can consequently contaminate the products.

(Sanna et al., 2018) opined that, though conventional microbiological techniques require specific skills, long execution and analysis times, they are the most widely used method to assess hygienic quality. According to Shama & Malik (2013) alternative methods including the adenosine triphosphate (ATP) bioluminescence assay, based on the measurement of levels of ATP present on the surface for measuring environmental cleanliness have been proposed in the last decade. Bioluminescence test exploits the chemiluminescence properties of luciferin-luciferase reagent, which reacts with any ATP residue present on a substrate, emitting light and measuring the presence of organic matter. According to Osimani (2014) although ATP bioluminescence technology cannot replace conventional microbiological analyses for the determination of microbial load on food contact surfaces, it has proved to be a potential device for the concurrent evaluation of surface cleanliness, for verifying the correct implementation of SSOP.

Tršan et al. (2019) reported that critical points are the sites that correspond to the utmost microbiological risk for aseptic practice. Surface samples and air samples must be taken at that stage where the product is mostly exposed and the risks are greatest. (Hassan et al., 2017) reported that the occurrence of organic substances on food contact surfaces can support the clinging of microorganisms and produce a food safety risk. Therefore, appropriate surface cleaning and sanitizing are critical safeguards against foodborne illness. Left over ATP detected on food contact surfaces are linked with cleanliness of food contact surface. Effective sanitizing can be accomplished after systematic cleaning to eliminate food residues and other organic materials.

Rapid and objective response to surface cleanliness is of paramount importance for fishery value chain to achieve the recommended hygiene level. Rapid hygiene monitoring systems (HMS) utilizing ATP-bioluminescence have been developed by Kyriakides (1992) and have demonstrated usefulness as tools to evaluate the cleanliness of food contact surfaces. According to Flowers et al. (1997), although ATPbioluminescence assay is considered as a quick and objective method for assessing cleanliness, it appears to be inadequately standardized at both the national and international level. HMS systems rely on the luciferin luciferase enzyme complex and the measurement of generated light (bioluminescence) for the detection of ATP.

The present study focused on those areas in Value Chain of Aliyar reservoir to improve quality and safety of fish through systematic approach that can ultimately leads to the overall development of the reservoir fishery. The study aimed to assess the possibility of using the bioluminescence technique as a rapid diagnostic method directly to validate the hygienic quality of the most critical zones in fishery value chain in Aliyar reservoir, where the last point is ready to eat (RTE) fish. The study gives an idea about the most significant contributor to organic debris among the 33 contact surfaces considered in the value chain, which needs more attention in maintaining food safety. The study also investigated the effectiveness of recommended Sanitation Standard Operating Procedure (SSOP) over the existing method.

Materials and Methods

The most prevalent value chain in Aliyar reservoir area consisting of four major components viz., harvesting unit (coracle), auction cum assortment area (AAA), pre-processing area (PPA), cooking and marketing area (CMA) were taken for the present study. A total of three surface contacts from each harvesting unit, 10 from auction cum assortment area, 10 from pre-processing area and 10 from cooking and marketing area were selected for hygiene monitoring. The sampling was done two hours after starting the process before and after following suggested SSOP in each critical area. From each sampling surface, two adjacent areas were selected, one area where SSOP was not followed, but normal cleaning process (NCP) was done. In adjacent area, SSOP was followed as per the suggested protocol given below.

Sanitation Standard Operating Procedures (SSOP):

1. Remove organic remnants on the surface by scraping out.
2. Pre-rinse the surface to remove small organic remnants and to wet the surface for the application of detergent.
3. Apply a food grade detergent on the food contact surfaces.
4. Wash surfaces with potable water to eliminate all detergent residues.
5. Apply sanitiser, Sodium hypochlorite (bleach liquor) in different food contact surfaces. The concentration and the contact times were as per Hassan et al. (2013). The concentration of Sodium hypochlorite used on contact surfaces other than Floor, wall, worker's hand and fish was 50ppm. Concentration was 200 ppm for floor and wall and 20 ppm for worker's hand. Fish was not given any additional wash. The contact time given for the sanitiser was 20 minutes.

Two hours after the actual operation has started, from the pre-determined contact surface, an area of 25 cm^2 were swabbed and subjected to ATP-bioluminescence measurements to assess the hygiene status using ATP hygiene monitoring system (Hygiena SystemSURE Plus™, California, USA). The measurement procedure was performed by following the instructions of the manufacturers of the swabs. The time and date of the instrument was set to ensure its normal operation and accuracy. Total testing time including the reading did not exceed 45 sec. The results are given in relative light units (RLU). According to the instructions given by the manufacturer (Hygiena SystemSURE Plus™), ATP-B samples were collected with swabs. Selected surfaces were swabbed in a zigzag model, rotating and applying little pressure. Sample was transferred aseptically to the ultra-snap tube. Test tube was held firmly and bulb was bent to and fro till snap valve is broken. Then, bulb was squeezed to expel the liquid completely.The sample collection tip was soaked in liquid by gently shaking for 5-10 sec. The ultra-snap tube with sample was then inserted into the luminometer, the lid was closed and the reading was taken using the instrument. The ultra-snap tube containing an ATP bioenzyme, D-luciferin, was then added to the sampler to react with the swab taken and convert ATP into AMP (Adenosine monophosphate). The higher the RLU results, higher the contamination level in the sample.

Data on organic debris observed with and without employing SSOP from various fish contact surfaces pertaining to the four different critical domains in the value chain was analysed using paired t-test (Snedacor & Cochran, 1991) to find out the efficacy of SSOP procedure. Further, analysis of variance (ANOVA) was employed to compare the extent of organic load across the various contact surfaces within each domain. Prior to performing the analysis of variance, the test of normality and homogeneity in the data was ensured through Shapiro's test and Levene's test. Data on organic load from different contact surfaces observed with and without SSOP implementation was also subjected to principal component analysis (PCA) as per Morrisson (1990) to study the reduction in bacterial load and the critical contributing contact surface. All statistical analysis was performed using R software.

Results and Discussion

Cleaning removes dirt and organic remnants, which support the proliferation of specific spoilage microorganisms and pathogens from a surface, reducing food poisoning, fish spoilage and attraction of pests. The presence of the spoilage bacteria in fish value chain is generally due to an inadequate program of cleaning, sanitation, and maintenance. Insufficient cleaning leaves behind fish residues on contact surfaces that support the growth of potential spoilage organisms that recontaminate the fish. Residues might result from incomplete or neglected manual cleaning. Cleaning reduces contamination and helps to keep products safe to eat.

The organic residues on food contact surfaces (FCS) of the value chain can support the attachment of microorganisms including pathogens and create a food safety risk. Therefore, appropriate surface cleaning and sanitizing are crucial safeguards against foodborne illness. The organic debris load without implementation of SSOP observed from three different fish contact surfaces (FCS) were identified from the harvesting unit (coracle) of the fishery value chain and compared using ANOVA. Prior to performing the analysis of variance, the test of normality and homogeneity in the data was ensured through Shapiro's test and Levene's test. It was found that the three contact surfaces namely workers hand, floor and fish studied, varied significantly from each other with respect to organic debris load ($p<0.01$). The maximum load was recorded from worker's hand (Table 1).

Table 1. Organic debris load as ATP bioluminescence in harvesting area following NCP & SSOP

Cleaning process	ATP bioluminescence as RLU (Mean ± SE)		
	Workers hand	Floor	Fish
NCP	1825.25 ± 1.47[a]	918.25 ± 1.53[b]	618.12 ± 4.00[c]
SSOP	718.12 ± 1.54[a]	215 ± 1.58[b]	208.5 ± 1.46[b]

Values bearing different superscripts differ significantly (p<0.01)

Similarly, when the bacterial load with the implementation of SSOP intervention present in the contact surfaces studied within the harvesting area was compared, it was found that it varied significantly (p<0.01) with workers hand being significantly different from floor and fish. Further around tenfold reduction in organic debris load from workers hand was achieved after SSOP adoption and around fourfold decrease in case of floor.

The organic debris load observed from ten different contact surfaces, which were identified from the auction cum assessment area (AAA) of the fishery value chain was compared using ANOVA. Prior to performing the analysis of variance, the test of normality and homogeneity in the data was ensured through Shapiro's test and Levene's test. It was found that the contact surfaces namely platform, krate, balance, basin, showel, wall, floor, worker's hand 1, worker's hand 2, and fish studied varied significantly from each other with respect to organic debris load (p<0.01). The post-hoc tests grouped these variables into 8 groups. Showel and wall did not vary significantly as well as the variables worker's hand 1 and floor did not vary among them significantly. The maximum bacterial load was observed from floor of auction cum assortment area (Table 2).

Similarly, when the organic debris load after SSOP intervention in the contact surfaces studied from AAA was compared, it was found that the contact surfaces varied significantly (p<0.01). Post-hoc tests were performed, which grouped the surfaces into 9 groups. The analysis indicated a drastic reduction in the organic debris load from the krate and wall in AAA. Further around 4-fold reduction in organic debris load was achieved after SSOP adoption from platform and floor.

The organic debris load observed from ten different contact surfaces, which were identified from the pre-processing area (PPA) of the fishery value chain was compared using ANOVA and is presented in Table 2. Table 2 also point out that the contact surfaces namely platform, krate, balance, basin, knife, cutting board, worker's hand 1, worker's hand 2, floor and fish studied, varied significantly from each other with respect to organic load (p<0.01). The post-hoc tests grouped these variables into 8 groups. The Knife and worker's hand 2 did not vary significantly. The variables worker's hand 1 and basin also did not vary among themselves significantly. The maximum organic load was observed from platform of PPA and minimum load from knife (Table 2).

Similarly, when the organic load after SSOP intervention in the contact surfaces from PPA was compared with NCP, it was found that the contact surfaces varied significantly (p<0.01). Post- hoc tests were performed, which grouped the surfaces into 8. The analysis indicated that the adoption of SSOP could bring about moderate reduction in the organic load in platform (50%) and 3-fold decrease in workers hand. This indicates that crucial point of the value chain is the area, where cutting and cleaning of fish happens, which needs more attention as it is having more organic load that can affect quality and safety of fish.

The organic debris load, observed from ten different contact surfaces, was identified from the cooking and marketing area (CMA) of the fishery value chain compared using ANOVA. It was found that the contact surfaces namely platform, krate, balance, basin, knife, serving table, serving plate, RTE fish, workers hand and floor varied significantly from each other with respect to bacterial load (p<0.01). The post-hoc tests grouped these variables into seven groups. Platform, krate and basin did not vary significantly as well as the variables balance and serving table did not vary among themselves significantly. Maximum bacterial load was observed from floor of CMA and minimum load from workers hand. (Table 2).

Table 2. Organic debris load as ATP bioluminescence in contact surfaces of three domains *viz.*, AAA, PPA and CMA following NCP & SSOP

Auction cum assortment area (AAA)	ATP bioluminescence as RLU (Mean ± SE)									
	Platform	Krate	Balance	Basin	Showel	Wall	Floor	Workers hand 1	Workers hand 2	Fish
NCP	3207.87 ± 8.70^{b}	2814 ± 11.88^{c}	1916.12 ± 36.00^{e}	1014.75 ± 18.71^{f}	910.75 ± 10.52^{g}	2114.75 ± 34.98^{d}	5836.25 ± 19.18^{a}	816 ± 12.65^{g}	726.75 ± 12.28^{h}	717.88 ± 22.14^{h}
SSOP	755 ± 2.98^{b}	328.25 ± 0.50^{e}	464.25 ± 1.23^{c}	297.38 ± 1.04^{f}	266 ± 1.54^{g}	189 ± 1.33^{i}	1427.5 ± 2.50^{a}	208 ± 1.06^{h}	189.38 ± 4.28^{i}	415 ± 1.20^{d}
Pre-processing area (PPA)	Platform	Krate	Balance	Basin	Knife	Cutting board	Workers hand 1	Workers hand 2	Floor	Fish
NCP	2404.5± 41.73^{b}	1908.12± 27.95^{c}	1399.62 ± 33.02^{e}	999.25 ± 18.54^{f}	782 ± 10.26^{g}	1568.12 ± 36.34^{d}	914.12 ± 11.13^{f}	888± 8.29^{f,g}	7533 ± 50.84^{a}	411.62± 8.02^{h}
SSOP	1212± 1.37^{a}	365.5 ± 0.66^{e}	214 ± 0.94^{h}	315.50 ± 0.81^{f}	286.75 ± 1.09^{g}	436 ± 2.94^{c}	318.62 ± 0.85^{f}	326 ± 1.42^{f}	1080.38 ± 9.43^{b}	398 ± 1.81^{d}
Cooking and marketing area (CMA)	Platform	Krate	Balance	Basin	Knife	Serving table	Serving plate	RTE fish	Workers hand	Floor
NCP	1408.87 ± 20.77^{b}	1367.87 ± 20.50^{b}	1172 ± 8.00 ^{c}	1358.5 ± 10.08^{b}	930.5 ± 11.31^{g}	1088.62 ± 16.17^{h}	888 ± 8.89^{e}	599.25 ± 4.54^{f}	508 ± 1.42^{g}	6186.62 ± 9.18^{a}
SSOP	467.75 ± 3.49^{b}	314.75 ± 1.43^{c}	186 ± 1.16^{d,e}	156.12± 0.48^{f}	142.25 ± 0.83^{f,g}	163.25 ± 0.98^{e,f}	115 ± 1.15^{h}	205 ± 0.77^{d}	128 ± 0.95^{g,h}	1148 ± 8.58^{a}

Values bearing different superscripts differ significantly ($p<0.01$)

Similarly, when the organic debris load after SSOP intervention present in the contact surfaces studied from CMA was compared, it was found that the contact surfaces varied significantly ($p<0.01$). Post-harvest tests were performed, which grouped the surfaces into 8 groups. The analysis indicated that adoption of SSOP could bring about 5-fold decrease in the organic debris load in floor and 3-fold decrease in platform.

The study points out that ATP-bioluminiscence assay following suggested SSOP significantly decreased RLU compared with those following NCP. Hassan et al. (2017) followed same reference pattern and reported ATP-bioluminescence assay (ATB-B) as an efficient rapid test for detecting surface hygiene and cleanliness in fish processing industry as it is competent in detecting ATP from organic residues including pathogenic and non-pathogenic microbes.

As claimed by Sherlock et al. (2009), chemical tests for ATP may provide supplementary information on effectiveness of cleaning and permit identification of environmental surfaces that may necessitate further cleaning or SSOP amendments. By taking into consideration of ATP measurements before and after cleaning procedures, Anderson et al. (2011) raised important concerns on the consistency of execution and management of SSOP. Alternatively, as reported by Boyce et al. (2009), ATP readings played a crucial role in providing quantitative evidence of enhanced cleanliness of high risk areas after the execution of an intervention plan.

Data on organic load, observed from different contact surfaces of critical areas in fish value chain *viz.*, harvesting area, auction cum assortment area and cooking & marketing area without following SSOP, was subjected to principal component analysis (PCA). The aim was to find out the significant contributor to organic debris i.e. the contact surfaces along the value chain, which needs most attention in maintaining food safety. The current study concentrated on 33 contact surfaces along the value

Table 3. Fish Contact surfaces in critical area and code used for statistical analysis

Code	Contact surface (Harvesting area)	Code	Contact surface (Pre-processing area)	Code	Contact surface (Cooking and marketing area)
a	Workers hand	n	Platform	x	Platform
b	Floor	o	Krate	y	Krate
c	Fish	p	Balance	z	Balance
Auction cum assortment area		q	Basin	aa	Basin
d	Platform	r	Knife	ab	Knife
e	Krate	s	Cutting board	ac	Serving table
f	Balance	t	Workers hand 1	ad	Serving plate
g	Basin	u	Workers hand 2	ae	RTE fish
h	Showel	v	Floor	af	Workers hand
i	Wall	w	Fish	ag	Floor
j	Floor				
k	Workershand1				
l	Workers hand 2				
m	Fish				

chain. The various contact surfaces selected in each critical area and code used in the analysis are listed in Table 3.

The sampling adequacy of the data was tested using Kaiser-Meyer-Olkin statistic and the redundancy in variables was checked using Bartlett's test of sphericity. The PCA generated six PC's, which could explain 74% variability in the data.

Fig. 1 gives the contribution a set of identified variables to various six dimensions or PCs. The reference line, which is dotted corresponds to expected value, if the contribution was uniform. The variables above the dotted line are considered important in contributing to the first six dimensions extracted out of PCA.

Analysing the plot depicting the contribution of variables to the dimensions the following can be deduced:

- The fish contact surfaces in AAA namely balance, floor, and wall were important owing to their contributions to the PC dimensions. Variable 'h' pertaining to fish contact surface balance is heavily loaded on Components 1,3,4,5,6 and variable 'j', which denoted fish contact surface Floor is loaded on Components 1,4,5,6.
- Floor, cutting board, platform, balance and krate in the pre-processing domain of the value chain emerged important, which contributed to the organic debris. Platform and cutting board were the two fish contact surfaces, which have emerged out as most important as the variables pertaining to PP area. Platform loaded heavily on components whereas cutting board had its heavy loading on components 1,3,5,6. The contact surface balance denoted by variable 'p' loaded on components 1,2,3,4,6.
- The intervention points in cooking and marketing area (CMA), which needs attention were platform and floor. Both these variables represented by x and ag heavily loaded on components 4 and 5
- The analysis does not point out importance of three contact surfaces studied in the harvesting unit's (coracle) area as the variables a, b and c pertaining to them were plotted well below the reference line in contribution plot (Fig.1) generated through the PCA.

Fig. 2(a). gives the PC plot generated for Components 1 vs 2. The contact surfaces denoted by variables n and s, which denote platform and cutting board in P&P area, are pointed towards the same

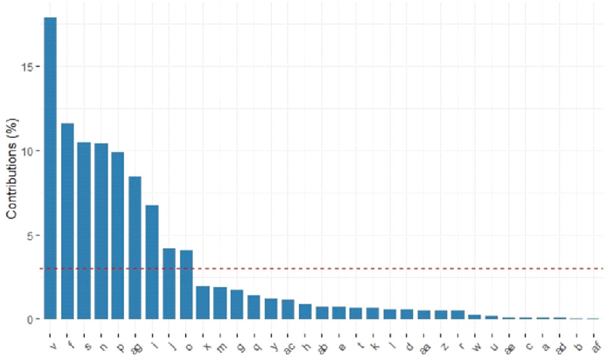

Fig. 1. Contribution of variables to Principal components 1 to 6

direction with small angles between them. This means that in P&P area, platform and cutting boards are closely related and contribute to the organic debris sequentially. The variable v, which denotes the floor of P&P area is drawn perpendicular to variables n and s, indicating the contamination in this contact surface very much more. As such the contamination level in floor of P&P area was found 3-fold in comparison with platform and 5-fold compared to cutting board. Also, the variables *p, f, i* and *o* are pointing in same direction in the plot indicating their close relationship. The variables *f* and *i* denote the contact surfaces wall and krate in A&A area. The fish, which arrives from the landing center is heaped on the auction floor and pushed aside the wall in the sales outlet before it gets distributed to consumers. Contact surfaces, krate and wall show more or less same level of contamination (Fig. 2(a)). The variables *p* and *o* denoted by balance and krate in P&P area also show similar level of contamination.

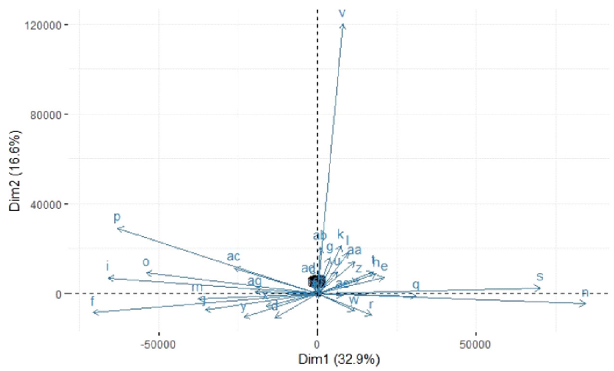

Fig. 2(a). PC plot Dim. 1 vs 2

Fig. 2(b). depicts the PC plot for components 1 vs 3. It highlights the importance of variables *ag, p, i, f, s* and *n*. The variable *ag* represents contact surface 'floor' of the C&M area and is pointed perpendicular to *f, s* and *n*. Floor has emerged as the CS with maximum contamination of this domain of value chain and needs attention.

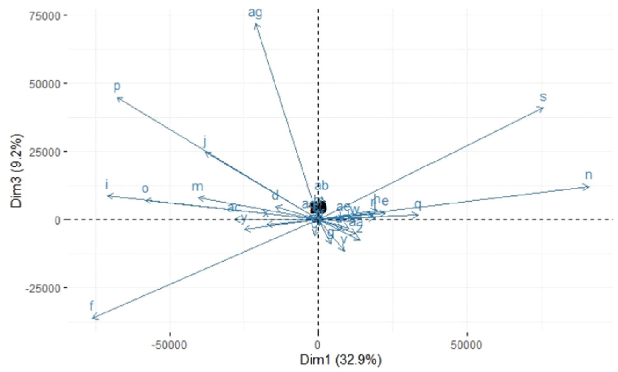

Fig. 2(b). PC plot Dim. 1 vs 3

Fig. 2(c), depicts PC plot for components 1 vs 4, where the variable *l* is seen prominent, which denotes the workers hand 2 from where the swab analysis was done. Workers are crucial nodes in a food value chain, and moderate organic debris load was recorded from this CS. Following SSOP measures strictly, this node can be controlled very effectively.

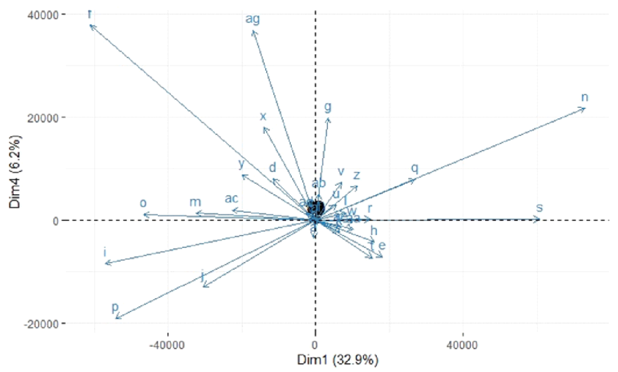

Fig. 2(c). PC plot Dim. 1 vs 4

Fig. 2(d). depicts PC plot for components 1 vs 5 and it highlights variables *x* and *y* apart from the important variables like *n, s, I, j, f, and p* which have been discussed previously. The variables *x* and *y* denote the contact surfaces, balance and platform in the C&M area, which have almost same level of moderate contamination issue. Fig. 2(e). give the PC plot for the dimensions 1 vs 6 again highlighting the close relation between variables *f* and *j*, which denote the floor and balance of A&A area.

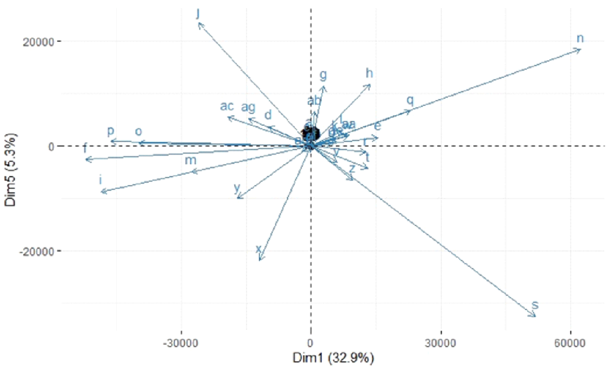

Fig. 2(d). PC plot Dim. 1 vs 5

These plots highlight the contribution of various contact surfaces in accumulating organic debris in the four major domains of the value chain. In some of the contact surfaces, the contamination is seen less pronounced. However, many contact surfaces need sanitization as they show moderate to high level of contamination and have emerged as crucial nodes in the value chain.

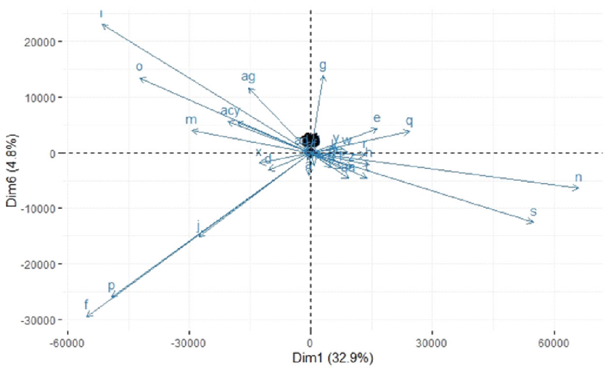

Fig. 2(e). PC plot Dim. 1 vs 6

Combined data on organic load observed, following NCP and SSOP from different contact surfaces belonging to harvesting area, auction and assortment area, pre-processing area and cooking & marketing area, were subjected to principal component analysis (PCA). The aim was to find out the effectiveness of the SSOP adoption in reducing the organic load, which inturn gives idea of cleanliness of selected fish contact surfaces.

There were a total of 33 contact surfaces studied along the value chain. The various contact surfaces and code used in the analysis were same as those given in Table 3.

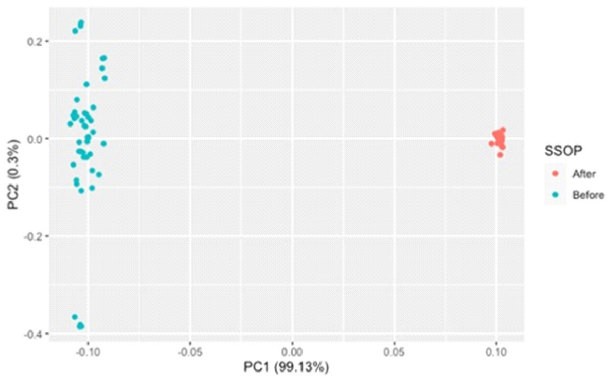

Fig. 3. PC plot of combined data

The sampling adequacy was tested using Kaiser-Meyer-Olkin statistic and the redundancy in variables was checked using Bartlett's test of sphericity. PCA extracted 2 components, which could explain more than 99.4% of the variance.

The PC plot (Fig.3) clearly separates the data of without adopting and with adoption of SSOP in FCS indicating the effectiveness of SSOP measures. The contribution plot (Fig.4) of variables to dimension 1 highlighted that among the FCS studied in the four critical zones, the floor of auction cum assortment area (AAA), pre-processing area(PPA) and the cooking and marketing area (CMA) were highly contaminated and needs special attention. At AAA and PPA, fish were placed even in floor, for the easiness of work. In such situations, the risk is much more. The other variables, which have emerged important as per the analysis were platform and krate in the AAA. As fish come in

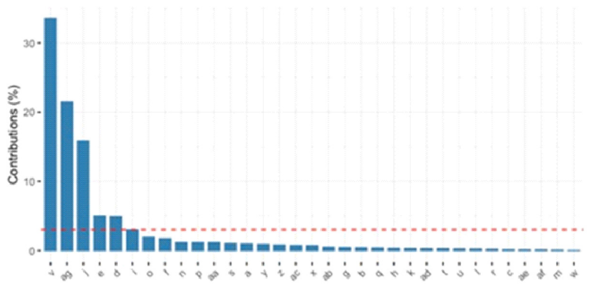

Fig. 4. Contribution of variables to Dim. 1

direct contact with the platform and krate, the hygiene of these FCS and its sanitation before, after and in periodical intervals is very essential and crucial as far as fish safety and quality is concerned. These are the critical points, which can result in the formation of high risk zones in the distribution chain and ultimately leads to food borne illness. Cunningham et al. (2011) opined that hygienic food contact surfaces are imperative in reducing the probability of food borne disease transmission and diffusion. Liu et al. (2014) reported that one of the main causes of recurrent food safety incidents was problems in food delivery pathways, thus supporting the results of current study.

The study exposed that the level of hygiene and sanitation of FCS in the value chain of Aliyar reservoir has to be improved. Proper hygiene and sanitation of FCS prevent microbial contamination and proliferation and thereby control fish spoilage and safeguards safety issues to a certain extent. Hence, appropriate hygiene and sanitation of FCS of value chain following SSOP is indispensable for food safety and quality, which ultimately food borne disease transmission and diffusion.

One of the probable causes of fish borne diseases is contamination along the distribution chain. In order to reinstate a low intensity of contamination, the contact surfaces should be thoroughly cleaned and sanitised before the start of work and periodically at regular intervals in between operations, following protocols suggested. Although not an alternative to conventional methods, the ATP-bioluminescence assay can be a practical mean to assess the competence of cleaning schedule in real time. Each facility should make out suitable reference values, depending on the procedure used and on the basis of the investigation of the data collected. The ATP-B assay provides a real time feedback, facilitating to increase the awareness of stakeholders by showcasing the result concurrently. It also helps to identify critical areas through which hazards can creep in and helps to take instant action in significant areas. Reservoir fisheries in Tamil Nadu caters to the nutritional requirements of the local population, who are settled in rural areas and have less access to food and nutritional supplements. Since Aliyar reservoir is a tourist spot, which attracts many tourists, who prefer ready to eat fish from the local shops, it is very essential to provide safe fish product. The study has potential for the real time monitoring of food contact surface hygiene and monitoring, for the verification of SSOP within an HACCP plan, and for the execution of corrective action against poor hygiene. The study also provides a diagnostic support for crafting consistent and complete strategies for guiding the systematic progress of the reservoir fishery to improve the quality and safety of the produce.

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