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ASSESSMENT OF QUALITY AND ADULTERATION OF FISH MARKETED IN DISTRICT LAHORE, PAKISTAN

J. Shafi[1, *], Z. S. Mirza[1], R. Yaseen[2], S. Y. Khan[2, **], S. Salim[1] and A. Tariq[1]

[1]Fisheries Research and Training Institute, Lahore, Pakistan
[2]Department of Zoology, Government College University, Lahore, Pakistan
Corresponding Authors' Email: *javairiamalik@gmail.com; **shafaatyarkhan@gcu.edu.pk

ABSTRACT

This study was conducted to determine the quality of fish sold in local markets of Lahore district, Pakistan and identify the potential health risks posed to consumers. Samples (54) of five different fish species: *Labeo rohita* (n=14), *Cirrhinus mrigala* (n=3), *Orochromis niloticus* (n=17), *Wallago attu* (n=3), and *Rita rita* (17) were randomly collected from the markets situated in four different zones of Lahore to determine their chemical, microbial and sensory quality. Absence of formaldehyde in the water rinsed from the surface of the fish samples ruled out the possibility of fish adulteration with this toxic preservative. Natural level of formaldehyde was greater than the permissible limit of 5 $mg \cdot kg^{-1}$ in 3.70% of the fish samples and varied from 1.73 ± 0.23 $mg \cdot kg^{-1}$ in *O. niloticus* to 2.43 ± 0.37 $mg \cdot kg^{-1}$ in *R. rita*. Content of total volatile basic nitrogen (TVB-N) was higher than the permissible limit of 25 $mg \cdot 100\ g^{-1}$ in 64.82% of the fish samples. Highest TVB-N content was found in *L. rohita* (36.09 ± 3.78 $mg \cdot 100\ g^{-1}$) while samples of *C. mrigala* showed the lowest content (26.30 ± 2.06 $mg \cdot 100\ g^{-1}$). Fish samples collected from Allama Iqbal Zone showed maximum TVB-N content followed by Data Ganj Baksh, Allama Iqbal and Wahga Zones. Highest total plate count ($30.93 \times 10^3 \pm 13.53 \times 10^3$ $cfu \cdot g^{-1}$) was recorded for samples of *O. niloticus* closely followed by *C. mrigala*, *R. rita and W. attu.* Total coliform and fecal coliform were higher than the permissible limit in 7.4% and 14.8% fish samples respectively. Total Quality Index of all the fish samples was higher than the borderline score of 12. In conclusion there is no possibility of fish adulteration with formaldehyde in the study area. Nevertheless, the high levels of chemical spoilage indicators and microbial contamination in a significant proportion of samples indicate poor hygienic handling of commercially available fish. Targeted training, enhanced hygiene, strict food safety enforcement, and regular monitoring are urgently needed in the fish supply chain to protect consumer health.

Keywords: Adulteration, fish spoilage, TVB-N, formaldehyde, microbial load, coliforms, sensory assessment

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INTRODUCTION

Food safety is directly related to a number of Sustainable Development Goals (SDGs) that focus on food security and public health. According to an estimate by the World Health Organization (WHO), one out of every ten people suffers from consumption of contaminated food globally. Moreover, about 420,000 people, including 125,000 children, lose their lives due to foodborne illnesses (WHO, 2024). Several laws and regulations addressing food safety are in place in the country, and provincial food authorities are also operating effectively. However, there is a lack of stringent regulations and enforcement mechanisms at the farm/ production level, leading to potential contamination risks from pesticides, fertilizers, and unhygienic practices (Rashid *et al.*, 2022).

Fish and seafood, being a source of high-quality protein and omega-3 highly unsaturated fatty acids (HUFAs), can play a key role in combating hunger and malnutrition (Maulu *et al.*, 2021). However, fish is one of the highly perishable food commodities and requires an efficient cold chain to minimize the spoilage. Fish spoilage is the result of complex processes including microbial, enzymatic and oxidative activities, each of which is exacerbated by handling practices and storage temperature maintained along the supply chain (Kruijssen *et al.*, 2020).

Different approaches based on sensory, microbial and chemical methods are used for assessment of fish quality. Sensory parameters including fish appearance, texture, odor, and flavor are based on human senses and are determined either through Quality Index Method (QIM) or grading schemes of European Union (EU) (Cardoso *et al.*, 2021). Microbial quality of fish is determined through estimation of total microbial load which is reported as total plate count (TPC) or total viable count (TVC). Presence and count of certain nonpathogenic bacteria including coliforms and members of Enterobacteriaceae are used as predictive indicators of presence of pathogenic microbes. Total Volatile Basic-Nitrogen (TVB-N), which refers mainly to trimethyl amine (TMA), dimethyl amine (DMA) and ammonia, is considered as an important chemical marker of fish spoilage (Toldrá and Nollet, 2024). Fish/ seafood mainly from marine origin contain significant amount of trimethyl amine oxide (TMAO) which is converted by microbial/ enzymatic action to trimethyl amine (Ashie *et al.*, 1996). In species that contain trimethyl amine oxidase (TMAOase), TMAO is also converted to dimethyl amine and formaldehyde (Dalgaard, 2003). The following reactions describe these chemical changes (Ashie *et al.*, 1996; Dalgaard, 2003).

$$\underbrace{XH_2}_{\substack{\text{Substrate}\\ \text{(Oxidized form)}}} + \underbrace{(CH_3)_3\,NO}_{\text{TMAO}} \rightarrow \underbrace{(CH_3)_3\,N}_{\text{TMA}} + \underbrace{A}_{\substack{Substrate\\ (Reduced\ form)}} + H_2O$$

$$\underbrace{(CH_3)_3\,NO}_{\text{TMAO}} \rightarrow \underbrace{(CH_3)_2\,NH}_{\text{DMA}} + \underbrace{HCHO}_{\text{Formaldehyde}}$$

In 2004, the International Agency for Research on Cancer (IARC) declared formaldehyde, one of the most commonly used food preservatives (Momtaz *et al.*, 2023), as a Group I carcinogenic compound for humans (IARC Working Group, 2006). Afterwards, its use as a food preservative has been banned in a number of countries (Nowshad *et al.*, 2018). According to a number of reports from various regions of the world, formalin (an aqueous solution of formaldehyde) is used by vendors along the supply chain to inhibit fish spoilage (Kaur *et al.*, 2024). Nevertheless, due to its natural production in the fish as shown in the above reaction, detection of formaldehyde adulteration becomes a challenge (Jinadasa *et al.*, 2022).

Due to the numerous health benefits associated with a fish/ seafood-based diet, combined with its very low per capita consumption compared to the world, several initiatives have been taken at the national level to increase the availability and consumption of seafood in Pakistan. However, the cold chain infrastructure in the freshwater fish supply chain is not well-developed, raising concerns about the quality of marketed fish (Patil *et al*., 2018). Food fish is, generally, transported and marketed using a bare amount of ice. This scenario, coupled with raised concerns about the fish adulteration with formaldehyde in developing countries, necessitates regular assessment of edible fish marketed in the country. The present study was, therefore, designed to assess the potential formaldehyde adulteration as well as chemical, microbial and sensory quality of fish marketed in the district Lahore, Pakistan, to determine its safety for the consumers.

MATERIALS AND METHODS

Sample Collection: Fish species were selected on the basis of their availability during the sampling period (October – December, 2024). Four zones of Lahore, including Allama Iqbal, Aziz Bhatti, Wahga and Data Ganj Baksh (Table 1, Figure 1) were selected for collection of fish samples due to their broad geographic and socioeconomic representation of the city's fish markets. The number of samples collected from each zone was proportionate to the density of fish retailers in that area to maintain sampling balance. Fifty-four (54) raw fish samples of five different fish species viz. *Labeo rohita* (n=14), *Cirrhinus mrigala* (n=3), *Oreochromis niloticus* (n=17), *Wallago attu* (n=3) and *Rita rita* (n=17) were purchased from retailer shops in the markets of Lahore using a probability sampling technique. These retailers procured their fish stock from the wholesale fish market situated near Mori Gate, Lahore. Immediately after purchase, fish samples were packed individually in sterilized bags, stored in ice boxes and transported to the Laboratory of Fisheries Research & Training Institute, Lahore, Pakistan. To provide a reference baseline for chemical and microbial parameters and sensory attributes in freshly caught fish, samples of *Oreochromis niloticus, Pangasius spp.*, *Labeo rohita*, *Cirrhinus mrigala* and *Ctenopharyngodon idella* were harvested from the production ponds of Fisheries Research & Training Institute, Lahore, Pakistan and analyzed using the same analytical methods as used for the marketed fish samples.

Sensory Analysis: For the assessment of sensory parameters of fish samples, Quality Index Method was adopted following Yu *et al.* (2017). A panel of three trained laboratory personnel was selected to score the marketed as well as fresh fish samples on the basis of their appearance, odor, texture and overall acceptability. A scale with a score ranging from 1 (lowest quality) to 5 (highest quality) was set for each parameter. The combined score of all sensory parameters was used to calculate Total Quality Index (TQI). For TQI, the highest possible score was 20, and a TQI score of 12 was set as borderline based on the scores set for individual sensory characteristics. Fish samples with TQI < 12 were considered unsuitable for consumption. The scores of the panelists for each sensory attribute of each fish sample were averaged to obtain a sample-level mean. The sample-level means were, then, aggregated by species and zones for reporting.

Chemical Analysis: For detection of formaldehyde use as a preservative, surface of whole fish samples was rinsed with 25 mL distilled water. The rinsate was collected in 100 mL beakers, mixed with 2 mL Nash reagent (Nash, 1953) and heated at 60 °C for 30 min. Appearance of yellow color in the solution was considered as positive test for the presence of formaldehyde. In the next step, fish samples were gutted, washed with distilled water, beheaded and deboned. Flesh collected from the dorsal muscles of the fish was homogenized in a lab blender for 1 min. The resulting fish mince (30 g) was homogenized with trichloro acetic acid (TCA, 7.5%, 60 mL) for extraction of formaldehyde and TVB-N. Formaldehyde content in the fish samples was determined by using Nash Reagent (Castell *et al.*, 1973; Nash, 1953). The suspension of fish mince in trichloroacetic acid solution was allowed to stand at room temperature for 1 h, mixed with distilled water to improve fluidity and centrifuged. For formaldehyde analysis, the pH of an aliquot (5.0 mL) of the supernatant was adjusted between 6.00 – 6.50 using 1 N solution of NaOH/ HCl. The extract was then kept at -20 °C for 1 h, mixed with 2.0 mL Nash reagent and heated on the water bath at 60 °C for 30 min. The absorbance of the resulting solution was immediately recorded at 415 nm. For quantification of formaldehyde in the fish mince's extract, formaldehyde standard solutions were prepared in the range of 0.1 mg/L to 20.0 mg/L using standardized formalin solution (≈37%).

Table 1: GPS locations of the sampling sites in four zones of Lahore

Zone	Latitude	Longitude	No. of samples
Allama Iqbal	31.46761	74.26661	19
	31.46659	74.23447	
	31.46981	74.24966	
	31.4617	74.30971	
Data Ganj	31.53094	74.31644	20
Bakhsh	31.54284	74.32036	
	31.57578	74.3096	
Wahga	31.58437	74.47579	3
Aziz Bhatti	31.5698	74.36503	12

For analysis of TVB-N, steam distillation method used by Sewwandi *et al.* (2016) was employed with slight modification. In the receiving flask, boric acid solution (25.0 mL, 4%) was added with a few drops of mixed indicator solution (bromocresol green/ methyl red; 3:1). An aliquot of the TCA extract (25 mL) was mixed with NaOH (10%, 6 mL) and distilled water (200 mL) in 250 mL distillation flask with immediate sealing of the distillation apparatus. Distillate (≈150 mL) collected in the receiving flask was titrated against standardized H_2SO_4 solution (0.02 N). A reagent blank was also run with each batch of samples. TVB-N was reported as $mg \cdot 100\ g^{-1}$ fish muscle.

pH of the fish muscle was determined by stirring the fish mince (≈5.0 g) with distilled water (50 mL) for 30 min. pH of the resulting solution was then recorded. Similar procedures were adopted for determination of formaldehyde and TVB-N content as well as pH of the freshly caught fish samples.

Microbial Analysis: Microbial quality of fresh and marketed fish samples was determined through assessment of Total Plate Count (TPC), Total Coliform (TC), Fecal Coliform (FC) and *E. coli* following the standard methods described in AOAC (2023) and BAM (FDA, 2023). Meat (50 g) was collected from the ventral muscles of fish samples and homogenized with sterile Butterfield's buffer solution (450 mL). Serial dilutions prepared from the resulting suspension were used to analyze microbial quality parameters. Total viable count was determined by mixing the dilutions with molten plate count agar and incubating at 35 °C for 48 h with subsequent counting of colonies on plates. For presumptive test of total coliform and fecal coliform, dilutions mixed with lauryl tryptose broth were incubated at 35 °C for 48 h and observed for formation of gas. Confirmatory test for total coliform and fecal coliform was performed using brilliant green lactose broth and *E. coli* broth respectively. Both of these broths were taken in separate test tubes and added with a loopful of suspension from gas positive tubes in presumptive test, with subsequent incubation at 35 °C for 48 h. For estimation of *E. coli* count, streak Levine's Eosin-methylene blue agar plates were streaked with a loopful of suspension from gas-positive tubes in *E. coli* medium. After incubation of the agar plates at 35 °C for 48 h, suspicious colonies (up to 5) were reinoculated into tryptone water and tested for indole production using Kovacs' reagent. (AOAC, 2023; FDA, 2023).

Statistical Analysis: Results for chemical, microbial and sensory parameters of marketed fish samples from four zones of Lahore and freshly harvested samples from fish ponds in Lahore were compiled using descriptive statistics. Species-wise comparisons and zones-wise comparisons were used to present and statistically analyze the data on fish quality, using one-way analysis of variance (ANOVA). For species-wise comparisons, data from all zones were pooled, and one-way ANOVA was performed to test differences among species. In the case of zone-wise comparisons, one-way ANOVA was performed on the quality parameters of fish samples pooled across all species to detect significant differences among the sampling zones of Lahore. Tukey's post-hoc test was applied to identify specific group differences following a significant ANOVA result. Due to interdependence among fish quality indicators, Pearson's correlation was used to identify significant correlation between chemical, microbial and sensory quality attributes of collected fish samples. All statistical analysis were two tailed and was conducted at significance level of .05 unless otherwise specified.

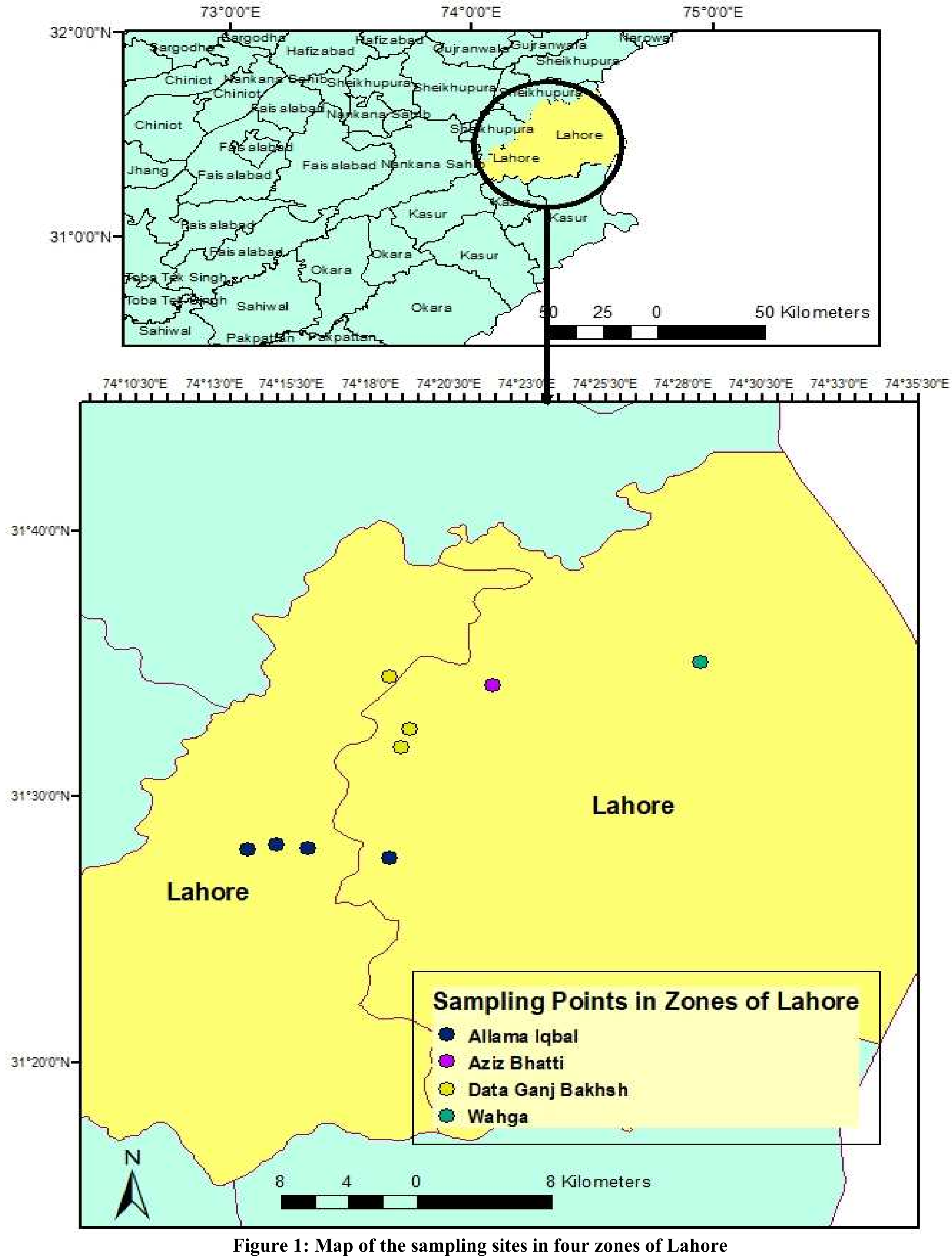

Figure 1: Map of the sampling sites in four zones of Lahore

RESULTS

Sensory quality attributes of the marketed fish samples varied among the species and the collection zone, however, the TQI did not decrease to the borderline limit of 12. Formaldehyde was detected in the fish muscles of freshly caught and marketed fish and was suggested to be of natural origin due to its absence in the surface rinsate. Key concerns identified in the study were high content of TVB-N, exceeding the permissible limit in a significant proportion of the samples and the contamination of samples with pathogenic coliforms.

Quality of Freshly Caught Fish: The chemical, microbial and sensory quality parameters of freshly caught samples of different fish species are presented in Table 2. Formaldehyde content of freshly caught samples ranged from 1.18 ± 0.11 mg·kg^{-1} to 3.63 ± 1.48 mg·kg^{-1}. The highest formaldehyde content (3.63 ± 1.48 mg·kg^{-1}) was found in samples of *C. idella* while *O. niloticus* and *Pangasius spp*. also showed content exceeding 3.0 mg·kg^{-1}. The level of TVB-N varied from 16.66 ± 0.59 mg·100 g^{-1} in *Pangasius spp*. to 21.40 ± 0.81 mg·100 g^{-1} in *C. mrigala*. The maximum value for muscles' pH (6.51 ± 0.07) was found for *L. rohita* while it ranged from 6.34 ± 0.04 to 6.41 ± 0.0 for *C. mrigala*, *C. idella* and *Pangasius spp*. The lowest muscles' pH was recorded for samples of *O. niloticus*. The highest TPC (3.1 x 10^3 ± 0.80 x 10^3 cfu·g^{-1}) was observed for samples of *Pangasius spp*. For the other four species, it ranged from 0.30 x 10^3 ± 0.13 x 10^3 cfu·g^{-1} to 0.81 x 10^3 ± 0.58 x 10^3 cfu·g^{-1}. The count for TC, FC, and *E. coli* was <3 for all freshly caught samples. Moreover, all the fish species gained maximum score for sensory quality parameters with a TQI of 20. Fish samples harvested from the ponds were also treated as the control group to provide a reference for quality parameters of freshly caught fish in comparison with marketed samples (Table 3, Figure 2-3).

Sensory Quality of Marketed Fish: Sensory quality of the samples of different fish species sourced from different zones is presented in Table 3. All the freshly caught fish samples gained maximum score for sensory quality parameters with a TQI of 20. Among the marketed fish, samples of *L. rohita* and *R. rita* received highest score for appearance. Score for texture decreased in the following order: *L. rohita* > *W. attu* = *C. mrigala* > *O. niloticus* > *R. rita*. Samples of *W. attu* and *R. rita* also received the lowest score for odor. For overall acceptability, samples of *W. attu* received the minimum score, closely followed by *R. rita*. Maximum TQI was observed for samples of *L. rohita* (19.28 ± 0.30) followed by *C. mrigala*, *O. niloticus*, *W. attu* and *R. rita*. However, TQI of all the samples was higher than the borderline limit of 12. While sensory parameters of samples showed statistically significant differences among different fish species, differences for fish samples from different zones were non-significant. Highest TQI was recorded for fish from Aziz Bhatti Zone (16.75 ± 0.70**)** followed by Allama Iqbal Zone and Data Ganj Baksh Zone. Lowest TQI (15.33 ± 1.7) was observed for samples from Wahga Zone.

Chemical Quality of Marketed Fish: Table 4 shows the content of formaldehyde and TVB-N along with pH in the samples of five fish species. The water rinsed from the surface of the fish samples did not show the presence of formaldehyde in the qualitative test. Formaldehyde content varied from 1.73 ± 0.23 mg·kg^{-1} (*O. niloticus*) to 2.43 ± 0.37 mg·kg^{-1} (*R. rita*) with a non-significant difference among fish species. Figure 2 shows the graphical presentation of chemical quality indicators in fish samples collected from different zones of Lahore along with the control group (freshly caught samples from the fish ponds). The highest formaldehyde content was recorded in the fish collected from Wahga Zone (4.10 ± 0.90 mg·kg^{-1}) followed by Allama Iqbal, Data Ganj Baksh, fish ponds, and Aziz Bhatti Zones (Figure 2a).

Highest TVB-N content was found in *L. rohita* (36.09 ± 3.78 mg·100 g^{-1}) whereas samples of *C. mrigala* showed the lowest content (26.30 ± 2.06 mg·100 g^{-1}). Level of TVB-N in fish muscles did not vary significantly among fish species. Fish samples collected from Aziz Bhatti Zone showed maximum TVB-N content followed by Data Ganj Baksh, Wahga and Allama Iqbal Zones (Figure 2b). Content of TVB-N recorded for fish from Aziz Bhatti Zone was significantly higher compared to Allama Iqbal and Data Ganj Baksh Zones. Lowest TVB-N content (019.18 ± 0.91 mg·100 g^{-1}) was recorded in the freshly caught samples.

pH of the fish samples varied from 6.12 ± 0.08 (*C. mrigala*) to 6.73 ± 0.05 (*O. niloticus*). Significant differences were observed in the pH of different fish species. pH of *C. mrigala* was significantly lower compared to *O. niloticus* and *R. rita*. When considering the zones, fish samples from Aziz Bhatti Zone showed the highest pH (6.82 ± 0.06) and those from Allama Iqbal Zone showed the lowest (6.48 ± 0.06). pH of fish from fish ponds, Allama Iqbal and Data Ganj Baksh Zones was significantly lower compared to Aziz Bhatti Zone (Figure 2c).

Microbial Quality of Marketed Fish: Table 5 shows the microbial quality parameters of the fish species. *O. niloticus* showed the highest Total Plate Count (30.93x 10^3 ± 13.53 x 10^3 cfu·g^{-1}) closely followed by *C. mrigala* and *R. rita*. The lowest TPC was recorded for samples of *W. attu* (2.92 x 10^3± 0.54 x 10^3 cfu·g^{-1}). A comparison of the microbial quality of fish species in terms of coliforms showed that the highest TC was recorded for *O. niloticus followed by L. rohita and C. mrigala* while the lowest was for *W. attu*. For FC, the highest count was shown by

Table 2: Quality parameters of freshly caught fish samples (Mean ± SE)

Chemical Quality	Fish Species				
	L. rohtia	***C. mrigala***	***O. niloticus***	***C. idella***	***Pangasius spp***
HCHO (mg·kg^{-1})	1.23 ± 0.130	1.18 ± 0.110	3.10 ± 0.451	3.63 ± 1.484	3.25 ± 0.057
TVB-N* (mg·100 g^{-1})	20.46 ± 3.674	21.40 ± 0.810	17.34 ± 1.431	20.47 ± 0.546	16.66 ± 0.589
pH	6.51 ± 0.070	6.41 ± 0.006	6.17 ± 0.007	6.34 ± 0.040	6.38 ± 0.043
Microbial Quality					
TPC* (cfu·g^{-1})	0.46 x 10^3 ± 0.198x 10^3	0.30 x 10^3 ± 0.134 x 10^3	0.81 x 10^3 ± 0.580 x 10^3	0.47 x 10^3 ± 0.207 x 10^3	3.1 x 10^3 ± 0.800 x 10^3
TC* (MPN·g^{-1})	<3	<3	<3	<3	<3
FC* (MPN·g^{-1})	<3	<3	<3	<3	<3
E. coli (MPN·g^{-1})	<3	<3	<3	<3	<3
Sensory Quality					
Appearance	5	5	5	5	5
Texture	5	5	5	5	5
Odor	5	5	5	5	5
Overall Acceptability	5	5	5	5	5
TQI*	20	20	20	20	20

* TVB-N: Total Volatile Basic Nitrogen; TPC: Total Plate Count; TC: Total Coliform; FC: Fecal Coliform; TQI: Total Quality Index

Table 3: Sensory assessment of marketed fish samples (Mean ± SE)

Species	n	Appearance	Texture	Odor	Overall Acceptability	TQI*
L. rohita	14	5.0 ± 0.0	4.86 ± 0.097^{a}	4.64 ± 0.169^{a}	4.78 ± 0.155^{a}	19.28 ± 0.304^{a}
O. niloticus	17	4.70 ± 0.114	3.47 ± 0.244$^{\mathbf{b}}$	3.88 ± 0.256^{ab}	3.65 ± 0.226^{b}	15.70 ± 0.668^{bc}
W. attu	3	4.33 ± 0.333	4.00 ± 0.0^{ab}	3.33 ± 0.667^{ab}	3.00 ± 0.577^{b}	14.67 ± 1.453^{bc}
C. mrigala	3	5.00 ± 0.0	4.00 ± 0.577^{ab}	4.67 ± 0.333^{ab}	4.67 ± 0.333^{ab}	18.33 ± 1.202^{ab}
R. rita	17	4.65 ± 0.119	2.76 ± 0.219^{b}	3.35 ± 0.270^{b}	3.35 ± 0.270^{b}	14.11 ± 0.568^{c}
Sampling Zone						
Fish Ponds (Control)	14	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	20.0 ± 0.0
Allama Iqbal	19	4.74 ± 0.104	3.68 ± 0.287	3.89 ± 0.275	3.95 ± 0.281	16.36 ± 0.837
Aziz Bhatti	12	5.00 ± 0.0	3.83 ± 0.271	4.00 ± 0.275	3.92 ± 0.260	16.75 ± 0.698
Data Ganj Bakhsh	20	4.65 ± 0.109	3.60 ± 0.255	3.95 ± 0.256	3.8 ± 0.257	16.0 ± 0.628
Wahga	3	4.67 ± 0.333	3.33 ± 0.882	3.67 ± 0.333	3.67 ± 0.333	15.33 ± 1.76

^{abc} Means with a different superscript in the same column are statistically significant for species and sampling zone (p<0.05).
*TQI: Total Quality Index

Table 4: Chemical quality indicators of marketed fish species (Mean ± SE)

Species	n	Formaldehyde (mg·kg^{-1})	Formaldehyde (Surface rinse) **	TVB-N* (mg·100 g^{-1})	pH
L. rohita	14	2.07 ± 0.437	ND***	36.09 ± 3.781	6.44 ± 0.043 ^{bc}
O. niloticus	17	1.73 ± 0.230	ND	33.05 ± 2.821	6.73 ± 0.054 ^{a}
W. attu	3	2.04 ± 0.850	ND	32.39 ± 12.046	6.6 ± 0.087 ^{abc}
C. mrigala	3	2.35 ± 0.494	ND	26.30 ± 2.064	6.12 ± 0.085 ^{c}
R. rita	17	2.43 ± 0.369	ND	27.65 ± 2.333	6.62 ± 0.057 ^{ab}

^{abc}Means with a difference superscript in the same column are statistically significant (p<0.05).
*TVB-N: Total Volatile Basic Nitrogen
**Qualitative determination of formaldehyde in water rinsed from fish surface
***ND =Not detected

samples of *C. mrigala* and lowest by *R. rita*. The *E. coli* count was found to be less than 3 in all samples.

Comparison of the fish microbial quality in different zones (Figure 3) showed that highest TPC was observed for samples collected from Wahga Zone (85.67

x10^3 ± 20.63 x 10^3 cfu·g^{-1}) followed by Allama Iqbal Zone (32.24 x 10^3 ± 13.47 x 10^3 cfu·g^{-1}), Data Ganj Baksh Zone (20.82 x 10^3 ± 8.34 x 10^3 cfu·g^{-1}), Aziz Bhatti Zone (3.4 x 10^3 ± 0.86 x 10^3 cfu·g^{-1}) and fish ponds (0.91 x 10^3 ± 0.304 x 10^3 cfu·g^{-1}). TPC of fish from fish ponds and Aziz Bhatti Zone was significantly lower compared to Wahga Zone (Figure 3a). For TC, the highest counts were observed in samples from the Allama Iqbal zone, while the lowest were recorded in samples from the fish ponds. In the case of TC, there was no significant difference among the zones, however, for FC, fish ponds, Aziz Bhatti Zone and Data Ganj Baksh Zone showed significantly lower count compared to Wahga Zone (Figure 3b-3c).

Correlation Analysis: Table 6 shows the results of Pearson's correlation among chemical, microbial and sensory quality parameters of marketed fish samples. Correlation analysis indicated significant positive correlation between formaldehyde content and TPC (r = 0.362, p<0.001). Formaldehyde content was also negatively correlated with sensory appearance of fish (r = -0.377, p<0.001). Significant negative correlation was found between pH and TC, FC, texture, and acceptability (r = -0.286, -0.289, -0.282, -0.341 respectively, p<0.05). A strong negative correlation was also found between pH and TQI (-0.353, p<0.001). In the case of microbial quality parameters, significant correlations were found between TPC, TC, and FC. Similarly, a significant correlation was observed among all sensory quality parameters, except for appearance and texture, which showed no significant correlation.

Table 5: Microbial quality indicators of marketed fish species (Mean ± SE)

Species	n	TPC* (cfu·g^{-1})	TC* (MPN·g^{-1})	FC* (MPN·g^{-1})	*E. coli* (MPN·g^{-1})
Permissible limit**		<5 × 10^5	<100	<10	<500
L. rohita	*14*	15.98 x 10^3 ± 10.310 x 10^3	89.3 ± 62.981(07)***	12.09 ± 5.703 ^{b} (07)	<3.0 (14)
O. niloticus	17	30.93x 10^3 ± 13.535 x 10^3	167.0 ± 155.559(10)	9.90 ± 3.994 ^{b}(11)	<3.0 (17)
W. attu	3	2.92 x 10^3± 0.536 x 10^3	23.60 ± 14.40 (1)	11.80 ± 8.20 ^{ab} (1)	<3.0 (3)
C. mrigala	3	29.65 x 10^3 ± 22.105 x 10^3	84.07 ± 40.890	23.2 ± 11.374 ^{a}	<3.0 (3)
R. rita	17	28.26x 10^3 ± 11.137 x 10^3	35.14 ± 17.369	7.95 ± 4.350 ^{b}	<3.0 (17)

^{abc}Means with a difference superscript in the same column are statistically significant (p<0.05).
*TPC: Total Plate Count; TC: Total Coliform; FC: Fecal Coliform
**Permissible limit specified by the International Commission on Microbiological Specifications for Foods (ICMSF)
***Values in round brackets indicate the number of fish samples for which the count was <3.0.

DISCUSSION

Sensory assessment of fish quality is considered fast and convenient, although it has also certain limitations. A highly negative correlation between formaldehyde content and fish appearance is an interesting finding of our study. TQI of any fish sample did not fall below the borderline limit of 12. TVB-N and formaldehyde content are the focus of ongoing research on seafood quality to determine health risks posed to consumers (Alkuraieef *et al.*, 2021; Gassem, 2019; Siregar *et al.*, 2021). According to the literature, formaldehyde content of fish varies widely depending upon the species and its natural content of TMAO, handling practices and adulteration (Jinadasa *et al.*, 2022). Due to this variability, European Union has not set a permissible limit for formaldehyde content in seafood. However, different countries have set their own permissible limit for the formaldehyde content of marketed fish. According to Sri Lankan Food Act, a limit of 5 mg·kg^{-1} has been prescribed for fish sold in Sri Lankan markets (Govt. of Sri Lanka, 2010). Malaysian Food Regulation Act, 1985 also specifies 5 mg·kg^{-1} as the allowable limit of formaldehyde in smoked fish (Noordiana *et al.*, 2011). Permissible limits of 60 mg·kg^{-1} and 10 mg·kg^{-1} for Gadidae fish and shellfish respectively are set by the Italian Ministry of Health (Bianchi *et al.*, 2007). According to the Chinese Ministry of Agriculture, maximum formaldehyde content of 10 mg·kg^{-1} is permissible in aquatic food products (Zhang *et al.*, 2015). Considering 5 mg·kg^{-1} as the permissible limit, we found that formaldehyde content was greater than this limit in 3.70% of the collected fish samples. In 24.07% of the collected samples, formaldehyde content was less than 1.0 mg·kg^{-1}.

We selected Nash reagent for screening of fish samples for formaldehyde due to its simplicity and cost effectiveness (Bhowmik *et al.*, 2020; Karlina *et al.*, 2024; Senthong *et al.*, 2021; Sewwandi *et al.*, 2016; Valadares *et al.*, 2021). Although the method may suffer from matrix interference, its results are comparable to other sensitive and high-tech methods (Valadares *et al.*, 2021). Several studies have investigated adulteration of food fish through qualitative detection of formaldehyde in the water rinsed from the fish surface (Haq *et al.*, 2017; Islam *et al.*, 2015). Islam *et al.* (2015) collected 939 fish samples from six districts of Bangladesh and reported that 22.68% of them were treated with formalin.

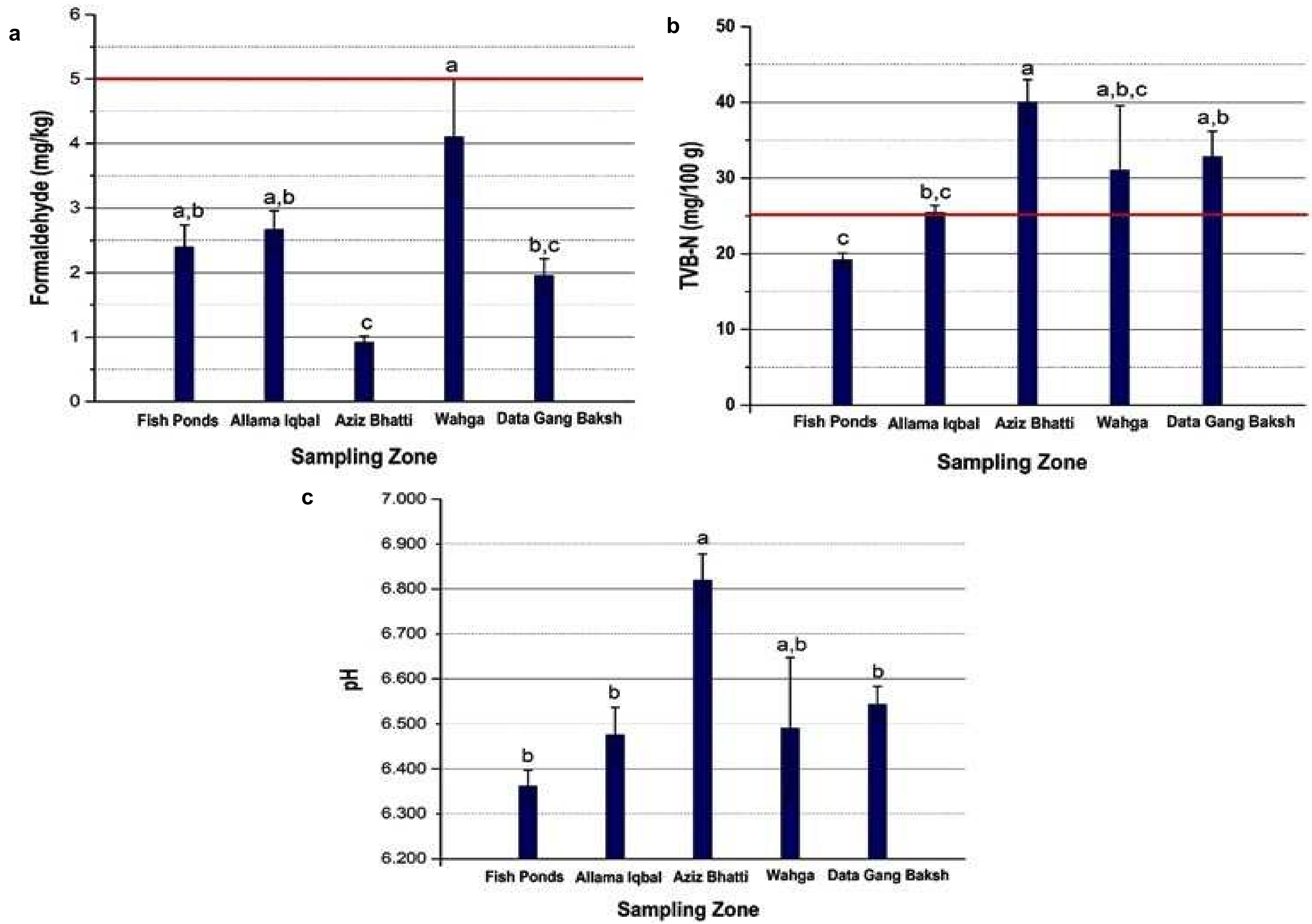

Figure 2: Chemical quality parameters of freshly caught and marketed fish. [a]: Formaldehyde content; [b]: Total Volatile Basic-Nitrogen (TVB-N); [c]: pH. Red line in [a] and [b] indicates permissible limit. Different small alphabets over the bars show significant differences among the areas

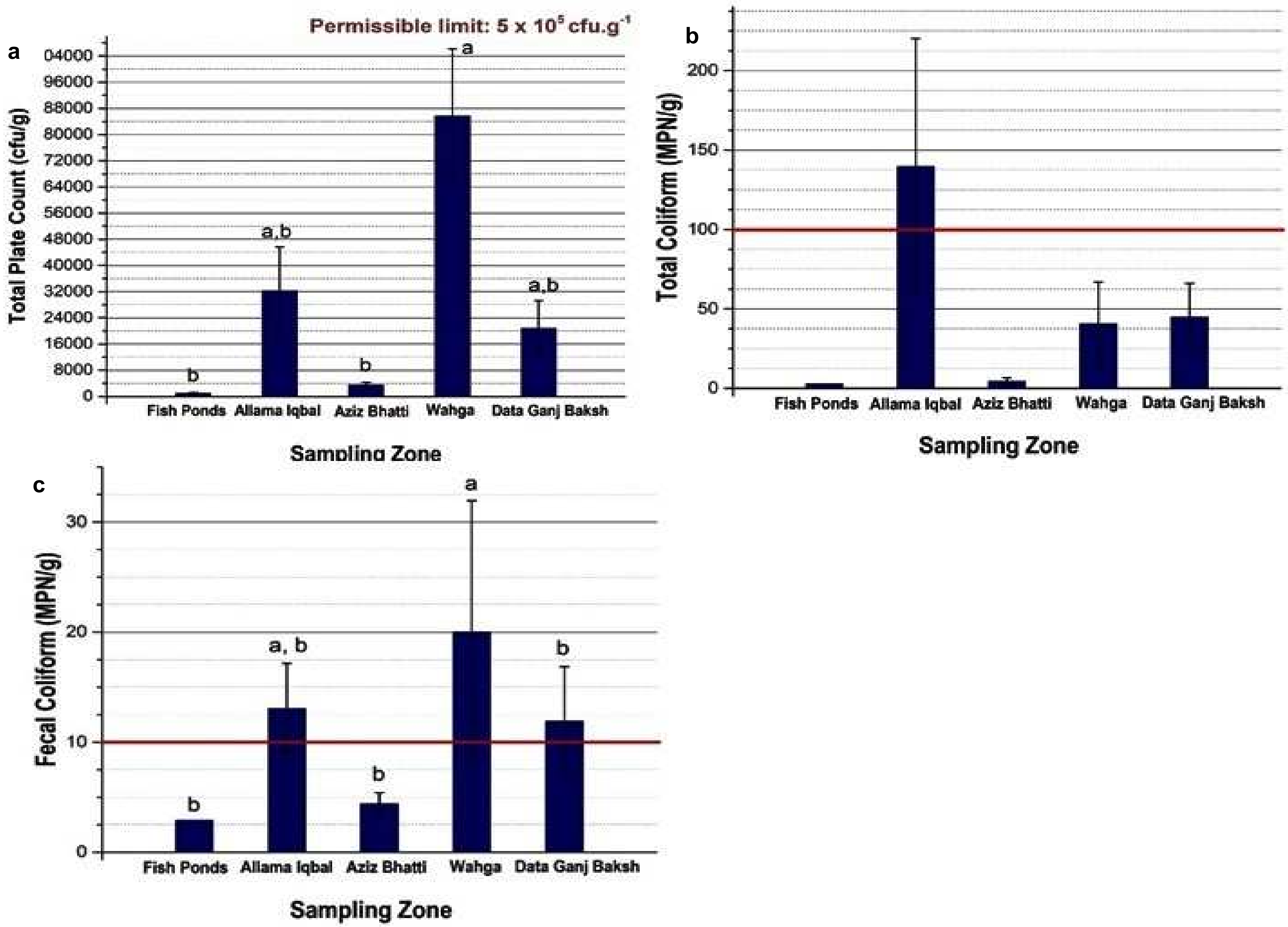

Figure 3: Microbial quality parameters of freshly caught and marketed fish. [a]: Total Plate Count (TPC); [b]: Total Coliform (TC); [c]: Fecal Coliform (FC). Red line in [b] and [c] indicates permissible limits. Different small alphabets over the bars show significant differences among the areas. In [b], the alphabets are not shown due to non-significant difference among the sampling areas.

Table 6: Correlation matrix for chemical, microbial and sensory quality parameters of collected fish samples

		TVB-N***	pH	TPC***	TC***	FC***	Appearance	Texture	Odor	Acceptability	TQI***
Formaldehyde	*r*	-0.208	-0.183	.362**	0.116	0.059	-.377**	-0.139	-0.190	-0.137	-0.224
	Sig.	0.132	0.186	0.007	0.403	0.669	0.005	0.315	0.169	0.324	0.104
TVB-N	*r*		0.155	-0.146	-0.198	-.291*	.307*	.277*	0.109	0.107	0.226
	Sig.		0.263	0.291	0.150	0.033	0.024	0.043	0.433	0.443	0.101
pH	*r*			-0.014	-.286*	-.289*	-0.230	-.282*	-0.254	-.341*	-.353**
	Sig.			0.920	0.036	0.034	0.094	0.039	0.064	0.012	0.009
TPC	*r*				.381**	.290*	-0.264	-0.265	-0.125	-0.096	-0.217
	Sig.				0.004	0.034	0.054	0.053	0.368	0.489	0.115
TC	*r*					.568**	-0.026	0.124	-0.022	0.106	0.073
	Sig.					0.000	0.853	0.370	0.875	0.445	0.598
FC	*r*						-0.127	0.036	-0.034	0.020	-0.010
	Sig.						0.360	0.795	0.807	0.887	0.945
Appearance	*r*							0.219	.488**	.538**	.594**
	Sig.							0.112	0.000	0.000	0.000
Texture	*r*								.350**	.410**	.681**
	Sig.								0.009	0.002	0.000
Odor	*r*									.945**	.901**
	Sig.									0.000	0.000
Acceptability	*r*										.931**
	Sig.										0.000

r: Pearson's correlation coefficient
*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)
***TVB-N: Total Volatile Basic Nitrogen, TPC: Total Plate Count, TC: Total Coliform, FC: Fecal Coliform, TQI: Total Quality Index

(Haq *et al.*, 2017) investigated formalin adulteration in six taxa of Small Indigenous Species (SIS) and reported that 50 out of 160 samples examined were contaminated with formalin. In our study, the presence of formaldehyde was not indicated in the water rinsed from the surface of the fish samples. Therefore, we concluded that detected formaldehyde content in fish muscles is unlikely to result from adulteration and likely to be produced naturally through decomposition of TMAO.

Asare-Donkor *et al.* (2018) determiend formaldehyde content in local and imported fish species available in markets of Kumasi Metropolis in Ghana and reported it to be 0.174 to 3.710 $mg \cdot kg^{-1}$. The authors suggested that edible fish available in the study area was safe for human consumption as its formaldehyde content was less than 5 $mg \cdot kg^{-1}$. Bhowmik *et al.* (2020) determined the formaldehyde content in edible fish sold in Kawran Bazaar, Dhaka city, Bangladesh. They reported 9.39 ± 3.39 $mg \cdot kg^{-1}$ to 32.57 ± 11.23 $mg \cdot kg^{-1}$ formaldehyde content in all collected fish samples and attributed it to natural breakdown of trimethyl amine oxide (TMAO). Rovina *et al.* (2020) reported formaldehyde adulteration (45.21–89.73 $mg \cdot kg^{-1}$) in *Lutjanus erythropterus*, *Euthynnus affinis*, *Crenidens indicus* and *Penaeus monodon* collected from market in Sabah, Malaysia. Jaman *et al.* (2015) have used spectrophotometric method for quantitative determination of formaldehyde in various fresh and marine fish samples collected from local wet market in Bangladesh. According to their findings, the fish samples collected from local markets showed higher formaldehyde content i.e. 1.4 and 7.35 $\mu g \cdot g^{-1}$. The freshly caught fish samples showed the natural concentration of formaldehyde with highest concentration of 2.6 $\mu g \cdot g^{-1}$ and 3.9 $\mu g \cdot g^{-1}$in thai koi from freshwater and loyitta fish from marine sources, respectively. The higher formaldehyde content in fish of local markets indicated fish adulteration with a carcinogenic preservative.

TVB-N content is considered an important indicator of fish quality. According to the European Commission Regulation No. 2074/2005, TVB-N permissible limits vary from 25-35 mg $N \cdot 100\ g^{-1}$ fish flesh for different species of marine origin (European Commission, 2008). However, EC regulation did not specify a limit for freshwater fish species. For the lean fish, 25 mg $N \cdot 100\ g^{-1}$ fish flesh has been considered as allowable limit (Giménez *et al.*, 2002). As all the fish species assessed in the present study were lean, we used the criterion of 25 $mg \cdot 100\ g^{-1}$ fish flesh for assessing fish quality on the basis of its TVB-N content. TVB-N content of 64.82% of the marketed fish samples was higher than the permissible limit of 25 $mg \cdot 100\ g^{-1}$. Moreover, TVB-N content was higher than 35 $mg \cdot 100\ g^{-1}$ for 31.48% of fish samples. Several earlier studies based on assessment of fish quality use TVB-N as an indicator of spoilage. Siregar *et al.* (2021) determined the chemical quality of *Rastrelliger sp*. available in markets of Jakarta, Indonesia. The authors reported that total volatile basic nitrogen (TVB-N) content of all the fish samples transported from the landing location to the supermarket was less than the permissible limit (30 $mg \cdot kg^{-1}$). However, TVB-N was higher than the permissible limit in 22% of the samples collected from traditional trading sites. Gassem (2019) reported 78.86 $mg \cdot 100\ g^{-1}$ sample TVB-N in salted fish samples collected from fish markets in Jazan and Abu-Arish in Saudi Arabia. Summers *et al.* (2017) determined trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) in commercial fish species available in markets of New Zealand. According to their reported data, TVB-N concentrations did not exceed the permissible limit (25-35 $mg \cdot 100\ g^{-1}$ muscles) set by the European Commission (European Commission, 2008).

pH of the muscles in freshly harvested fish remains in the range of 5.5 - 6.5 due to formation of lactic acid from anaerobic glycolysis (Ashie *et al.*, 1996). During postharvest storage and processing, pH tends to increase due to spoilage and accumulation of nitrogenous compounds (Mu *et al.*, 2021). However, the rate of fish spoilage, content of TVB-N and associated increase in pH can be largely reduced through an efficient cold supply chain. Kim *et al.* (2023) reported that pH of fillets prepared from largehead hairtail, Spanish mackerel and Chub mackerel increased gradually during 7 days storage period. Increase in pH was more pronounced for fillets stored at 20 °C compared to those kept at 4 °C. It is also noteworthy that freezing/chilling reduces the rate of spoilage but cannot completely inhibit it. In one of our previous studies (Z. Mirza *et al.*, 2023), it was found that pH of *O. niloticus* increased from 6.71 ± 0.0 to 7.01 ± 0.01 after 105 days of frozen storage. Afrin *et al.* (2021) observed that pH of fresh *L. rohita* fillets was 6.56 that increased to 7.12 after 14-week storage at -18 °C. In the present study, highest pH was observed for *O. niloticus* and lowest for *C. mrigala* among the marketed samples. Among the fresh samples, lowest pH was observed for *O. niloticus*. Moreover, correlation between TVB-N and pH was not significant, indicating that the observed pH values were not influenced by accumulation of nitrogenous compounds.

At the time of harvest, microbial load of fish reflects microorganisms present in the aquatic environment. Microbial load associated with warm water fish mainly comprises of mesophilic bacteria. Cold storage of the freshly caught fish as well as appropriate handling practices help to reduce the spoilage caused by the microbes (Dilmaçünal and Kuleaşan, 2018). Permissible limit for the microbial load of the freshly caught fish measured as total plate count is specified as 5×10^5 $cfu \cdot g^{-1}$ (ICMSF, 1986). Table 7 shows the distribution of fish samples based on their TPC count. About 9.0% fish samples collected from different zones showed total plate count in the range of 10^5 - 5 × 10^5 cfu·g^{-1}. However, for none of the samples, TPC higher than the permissible limit (5 × 10^5 cfu·g^{-1}) was observed. The permissible limit for total coliform is 100 MPN·g^{-1} and 7.4% fish samples showed coliform count higher than this limit in our study. For 14.8% samples, fecal coliform higher than the permissible limit of 10 MPN·g^{-1} was recorded. In none of the samples, *E. coli* was found to be higher than permissible limit (<500 MPN·g^{-1}). Significant correlation of TPC with TC and FC indicated high prevalence of coliforms in the total microbial load of the fish samples.

The presence of pathogenic coliforms in fish samples suggests contamination likely originating from unsanitary handling practices, poor hygiene at retail points, or the use of contaminated water during washing and storage **(**Patel *et al.*, 2014). Inefficiency in cold chain management and exposure to unhygienic environments during fish transfer and marketing may further contribute to its coliform load (Bartáková *et al.*, 2023). Studies based on the assessment of food microbial quality use coliform count as important indicator of food sanitary quality. In a previous study (Z. S. Mirza *et al.*, 2024), we found that total coliform and fecal coliform count was higher than the permissible limit in 9.30% and 53.45% fish samples collected from the markets of Lahore, Pakistan. Sarkar *et al.* (2020) reported that all the fish samples collected from markets of Dhaka (Bangladesh) were contaminated with *E. coli*. Mitiku *et al.* (2023) found that total coliform count in cooked and raw fish samples collected from upper Blue Nile watershed, Ethiopia, was 1.2 × 10^2 cfu·$g^{-1}$and 5.10 × 10^4 cfu·g^{-1} respectively. Chatta *et al.* (2018) found the microbial contamination of fish increased along the supply chain in Lahore, Pakistan, due to inappropriate handling conditions. According to their results, TPC of *L. rohita and C idella* collected from the main fish market was 1.22×10^6 cfu·g^{-1} and 1.12×10^6 cfu·g^{-1} respectively, compared to 2.25×10^6 cfu·g^{-1} and 4.82×10^6 cfu·g^{-1} for samples sourced from retailer shops. Siregar *et al.* (2021) found that *Rastrelliger sp*. collected from the markets of Jakarta were contaminated with *Escherichia coli* and level of contamination increased along the distribution chain. According to Gassem (2019), total bacterial count and coliform count of the salted fish samples from Saudi Arabian fish markets was found to be 2.81 to 4.72 Log_{10} cfu·g^{-1} and <1 Log_{10} cfu·g^{-1} respectively.

Table 7: Distribution of fish samples based on Total Plate Count levels

	Total Plate Count (cfu·g^{-1})					
Species	**<10^2**	**10^2 -10^3**	**10^3 -10^4**	**10^4 -10^5**	**10^5 - 5x10^5**	**>5x10^5**
L. rohita	0	10	2	1	1	0
O. niloticus	2	3	7	3	2	0
W. attu	0	0	3	0	0	0
C. mrigala	0	1	0	2	0	0
R. rita	0	6	5	4	2	0
Zone						
Allama Iqbal	1	8	4	3	3	0
Aziz Bhatti	0	5	7	0	0	0
Data Ganj Baksh	1	7	6	5	1	0
Wahga	0	0	0	2	1	0

Conclusion: The present findings rule out the possibility of fish adulteration with formaldehyde in the region. However, high levels of TVB-N and coliforms raise concerns about the fish quality marketed in the area. There is an urgent need to implement strict quality control measures at each step of the supply chain in the country to ensure provision of safe and high-quality fish for the consumers. There is need for strengthening the cold chain infrastructure in the fish supply chain and implementing targeted training programmes for fish handlers on fish safety and hygiene practices to minimize public health risks.

Authors Contributions: JS conceptualized the study, conducted experimental work related to fish chemical and sensory quality, analyzed the data and wrote the manuscript. ZSM conceptualized the study, provided technical support and reviewed the manuscript. RY conducted experimental work related to fish chemical quality. SYK provided technical support. SS and AT carried out the experimental work related to fish microbial quality.

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