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THE INFLUENCE OF WATER AND SEDIMENTS NITRITE CONCENTRATION ON CHEMICAL FISH MEAT COMPOSITION IN DIFFERENT AQUATIC ECOSYSTEMS

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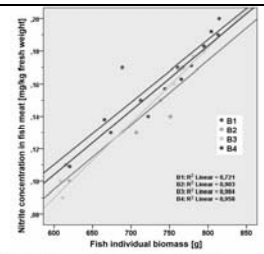
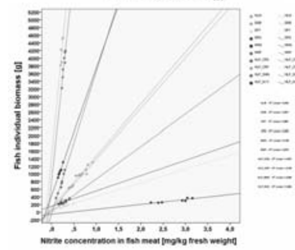
Pollution is a worldwide issue nowadays, fact that makes food safety a very popular concept that is encountered among the latest research in food chemistry field. The aim of the present study is to evaluate the chemical fish meat composition in terms of nitrites, in different ecosystems, in correlation with the concentration of nitrites from water and sediments. Therefore, samples of water, sediments and fish were collected from the following areas: Black Sea – Perişor (44°47'59.2"N - 29°16'14"E), Black Sea – Sfantul Gheorghe (44°52'51.3"N - 29°37'18.8"E), Danube Delta - Barcaz Lake (44°58'24"N - 29°12'54"E), Danube - Tulcea Port (45°10'53"N - 28°47'34"E), Danube – Galati Port (45°25'44"N - 28°3'25"E), Petrei Lake and Pond (43°3'9"N - 29°7'49"E), Malina Lake and Pond (45°24'27"N - 27°57'13"E) and also a recirculating aquaculture system. In Danube - Tulcea Port (0.2468mg/L), Danube – Galati Port (0.2083mg/L) and Black Sea – Sfantul Gheorghe (0.1988mg/L) were registered the highest concentration of nitrites in water. It has been concluded that in natural environment, there is not a strong direct correlation between nitrites concentration in fish meat, sediments and water. However, in an anthropogenic environment as RAS, there is a direct correlation between nitrites concentration in water and fish meat, during a production cycle, fact due to fish biomass long term exposure to a certain and relatively constant nitrite concentration.





INTRODUCTION

The important role of nitrogen to life on earth is well-known. The nitrogen cycle involves soil, plants and animals, which are tightly connected with the food chain.[6]

Nitrogen is an essential nutrient for all living organisms and it is found in proteins, nucleic acids, adenosine phosphates, pyridine nucleotides and pigments, but also nitrogen has been considered as one of the most important waste products affecting the quality of the receiving waters and their environment.[8-4] In the aquaculture environment, nitrogen is of primary concern as a component of the waste products generated by rearing fish.[8]

The production of nitrogen compounds by humans increased dramatically, especially during the second half of the twentieth century, therefore since the end of the 20^{th} century, humans have been adding more nitrogen compounds to the nitrogen cycle than all other sources combined, through the use of fertilizers or as a by-product of the combustion of fossil fuels.[15]

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In human alimentation, the potable water is the main source of nitrites, after vegetables, therefore legal limits were set for maximum concentration of nitrites in drinkable water of 0.5 mg/L.[1] Also, in Romania, the maximum allowable concentration of nitrites in drinking water is established by Law no. 311/28.06.2004 and 458/2002 which mentions a 0,3mg/L exceptional allowable value and 0 mg/L as allowable value. [23]

Nitrites and nitrates are also well-known food additives listed as official preservatives in the corresponding Communities Directives, but are considered harmful food additives.[6]

In his research, Hsieh, 2000 mentioned that once it reaches a pond environment, nitrogen can be distributed as follows: in gaseous state, settled in solids or suspended in the wastewater pond.[5] During the summer season, a specific amount of nitrogen is lost to the atmosphere at a higher rate, compared with other seasons. The total oxidized nitrogen consists of nitrate and nitrite nitrogen, both forms of nitrogen have been identified as growth-limiting nutrients, but have also been identified as toxic substances in high doses.[5]

As part of the nitrogen cycle, nitrites are naturally occurring ions and are ubiquitous in the environment. They are products of the oxidation of nitrogen by microorganisms in plants, soil or water.[15] Nitrites are natural components which result as intermediate products from bacterial processes of the nitrogen cycle in ecosystems, such as nitrification and de-nitrification. In natural waters, the concentration of nitrites is usually low (below 0.23 mg/L) and in oxygenated waters typically lower (below 0.005 mg/L).[12-18] In 2006, Kroupova et al. explained that elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metals, dyes and celluloid.[12]

While ammonium is a constituent present in a natural way in water, nitrates and nitrites are considered chemical parameters, being therefore represented by a different category of constituents. In case of recirculating aquaculture production systems (RAS), Timmons et al. (2002) mentioned that ammonia, nitrates, nitrites are highly soluble in water and they represent important water quality parameters that must be monitored and corrected if acceptable limits are exceeded.[8]

Among other factors, nitrites are considered primary in determining the quality of a water body, on which depends the reproduction and development of fish.[13] Nitrite toxicity to fish is strongly connected to the water quality and chemistry (temperature, cation, anion and oxygen concentration).[12]

In the aquatic systems, dead organic matter decomposes and turns into complex protein, which later is converted to ammonia, nitrite and less-toxic nitrate (which can cause mortalities at concentrations exceeding 1000 mg/L) and unlike carbon dioxide that is released to the air by diffusion or forced aeration, there is no effective mechanism to release the nitrogenous metabolite.[13-4-19]

Limits have also been imposed on nitrogen levels from wastewater that flows back into the environment, because without wastewater management systems, excess nutrients can destabilize ecosystems, cause anoxia, and kill large populations of aquatic life.[5]

As mentioned above, nitrites are natural components of the nitrogen cycle in ecosystems, and their presence in the environment is a potential problem due to the toxicity to animals.[17] Aquatic animals manifest a higher risk of nitrite intoxication compared with terrestrial animals. In case of fish found in water with high nitrite concentration, this compound can be actively taken up across the gill epithelium, fact that leads to high nitrite accumulation in the fish body fluids, plasma, gills, liver and brain.[12] Also, Kroupova et al. (2005) mentioned that high nitrites concentration in water can induce a variety of physiological disturbances in fish, which will generate toxicity.[17] Researches on fish revealed that nitrite is a disrupter of a variety of physiological functions as follows: ion regulatory, respiratory, endocrine and excretory processes.[12] In his book, Timmons (2002) stated that nitrite is toxic because it affects the blood hemoglobin's ability to transport oxygen. When nitrite enters the bloodstream, it oxidizes the iron in the hemoglobin molecule from the ferrous state to the ferric state. The resulting product is called methemoglobin, which has a characteristic brown color, hence the common name "brown-blood disease".[8] Methemoglobin forms slowly and spontaneously even in the absence of nitrite, thus fish blood typically contains a measurable amount of methemoglobin as follows: 3.6% for rainbow trout, 10.9% for prespawning pink salmon, 17.2% for channel catfish.[18] If the amount of methemoglobin in the blood does not exceed 50% of the total hemoglobin, the fish usually survive.[19] The 96-hour LC50s for nitrite-nitrogen typically range from 0.25 to 100 mg/L for fish as follows: 88 mg/L for the common carp, 7.1-44 mg/L for the channel catfish, 0.24-11 mg/L for the rainbow trout, 980 mg/L for the sea trout.[20] Nitrite concentrations in the blood plasma may reach 10 times the concentrations in the surrounding medium.[18] Taking up from ambient water, fish are able to accumulate nitrite in their bodies and reduce it to NO mainly by deoxygenated heme groups inside red blood cells.[7]

One of the most important factors influencing nitrite toxicity in fish is the chloride concentration in water and nitrite toxicity decreases with increasing chloride concentration.[12] The relationship between nitrite toxicity and chloride concentration is linear.[18] Nitrite ions are taken up by the chloride cells of the gills and the amount of nitrite entering the blood depends on the ratio of nitrite to chloride in the water, in that increased levels of chlorides reduce the amount of nitrite absorption.[8-19] Chloride ions have the same charge and are similar in size to nitrite ions, therefore chloride competes with nitrite for adsorption sites on the active carrier mechanism responsible for transporting environmental nitrite across the gill lamellae to the bloodstream.[20] Chloride cells excrete ammonia or H^+ ions for Na^+ ions and bicarbonate (HCO_3^-) for Cl^- ions.[21] Nitrites have an affinity for the branchial chloride uptake mechanism, the Cl^-/HCO3 – exchanger, thus, whenever nitrite is present in the ambient water, a part of the Cl^- uptake will be shifted to NO□ – uptake.[12] Some fish species have a higher speed of chloride uptake by the gills (pike, perch, rainbow trout) compared with others which have a lower speed of chloride uptake (common carp, tench).[21]

In the process of nitrification of ammonia to nitrate, the resulted nitrites may pose a problem in a recirculating system because they are being created on a constant basis, so fish are continually exposed to certain concentrations of this chemical, even though they are converted relatively quickly into nitrate by ozone and the nitrifying bacteria in a properly balanced biofilter.[8] In the aquatic production systems, the high fish densities and the high protein levels of fish feed, increase the content of nitrogenous compounds dissolved in the water, thus nitrite can reach toxic levels in fish within a few days, leading to physiological impairment and eventually death of the exposed animals.[9] According to Li et al. (2014) excessive nitrite can affect the development of nerve system or muscle in fish.[7]

The nitrites concentrations over the maximum limits can pose direct toxicity (through the oxidation of hemoglobin into methmeglobin) or indirect toxicity (through the participation in the formation of nitrosamines, which have mutagen and cancerigen action), but as well nitrites play an important role in eutrophication in the aquatic environment, which leads to taste and odor problems and even contribute to human health problems.[1-16] It must be also mentioned that nitrites presence in aquatic organisms is strongly related with the water matrix, the type of aquatic organism and also the environmental factors as temperature or atmospheric pressure.

Therefore, the aim of the present study is to evaluate the chemical fish meat composition in terms of nitrites in different ecosystems and also, to find if there are any correlations between nitrite concentration in fish meat and the concentration of nitrites in water and sediments.

MATERIAL AND METHODS

The sampling locations (Fig. 1 A and Fig 1 B) included both the natural environment (Black Sea Perisor- *MNP*- 44°47'59.2"N29°16'14"E, Black Sea Sf. Gheorghe- *MNG*- 44°52'51.3"N29°37'18.8"E, Danube Delta Barcaz Lake- *DDB*- 44°58'24"N29°12'54"E, Danube River Tulcea- *DPT*- 45°10'53"N28°47'34"E, Danube River Galati- *DPG*- 45°25'44"N28°3'25"E) and the anthropogenic environment (Petrei Pond- *HLP*- 43°3'9"N 29°7'49"E, Malina Pond- *HLM*– 45°24'27"N27°57'13"E and the recirculating aquaculture system pilot station of Aquaculture, Environmental Science and Cadastre Department of „Dunarea de Jos'' University of Galati- *RAS*- 45°26'41"N28°2'52"E).

Therefore, this research is conducted on two main water categories: fresh water (HLM, MoRAS, DPG, DPT, HLP, DDS, DDB) and salt water (MNP and MNG).

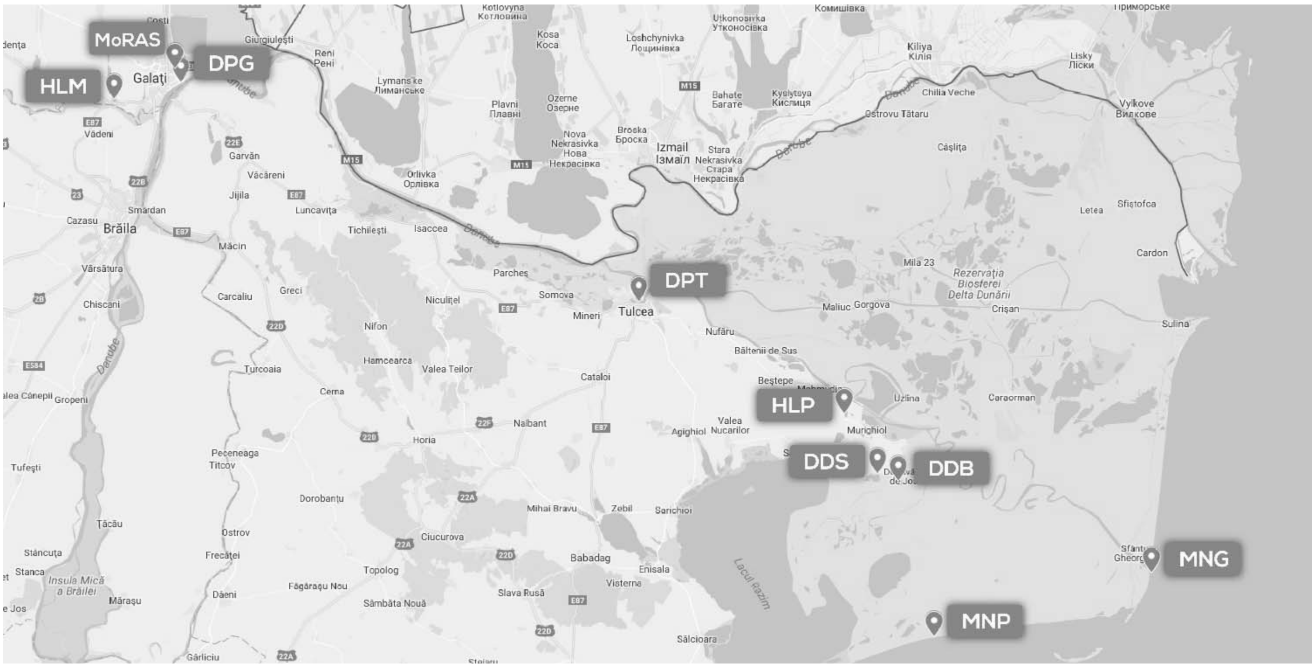

Fig. 1 A – Sampling locations from the natural environment.

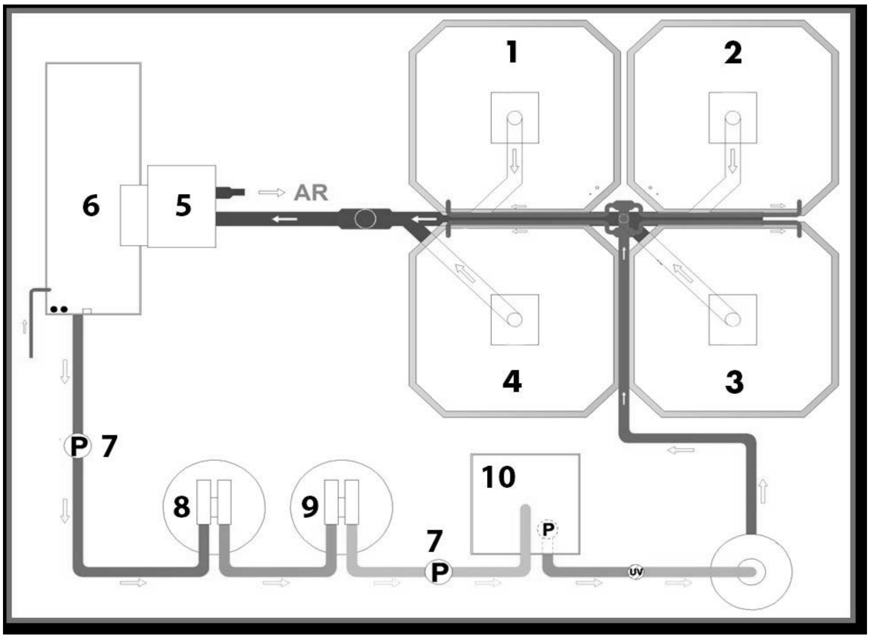

Fig. 1 B – The recirculating aquaculture system constructive design
(1, 2, 3, 4 - rearing units; 5 - drum filter; 6 - mechanical filter; 7 - recirculation pumps;
8 - sand filter; 9 - carbon filter; 10 - trickling filter).

Samples of water, sediments and fish were collected during spring season, April 2016. The water and sediment samples correspond to each of the points where fish catches were recorded. Different fish species were analyzed, as follows: shad (*Alosa immaculata,* Bennet, 1835) at *MNP,* shad (*Alosa immaculata,* Bennet, 1835) at *MNG*, prusian carp (*Carassius gibelio,* Bloch 1872) at *DDB*, prusian carp (*Carassius gibelio,* Bloch, 1872) at *DPT*, shad (*Alosa immaculata,* Bennet, 1835) at *DPG*, prusian carp- *CRS* (*Carassius gibelio,* Bloch, 1872) at *HLP*, catfish - *SMN* (*Silurus glanis,* Linnaeus, 1758), carp - *CRP* (*Cyprinus carpio,* Linnaeus, 1758), bighead carp - *NVC* (*Hipophthalmichtys nobilis,* Richardson, 1845), prusian carp (*Carassius gibelio,* Bloch, 1872) at *HLM* and russian sturgeon (*Acipenser gueldenstaedtii,* Brand & Ratzeburg, 1833) in *RAS*.

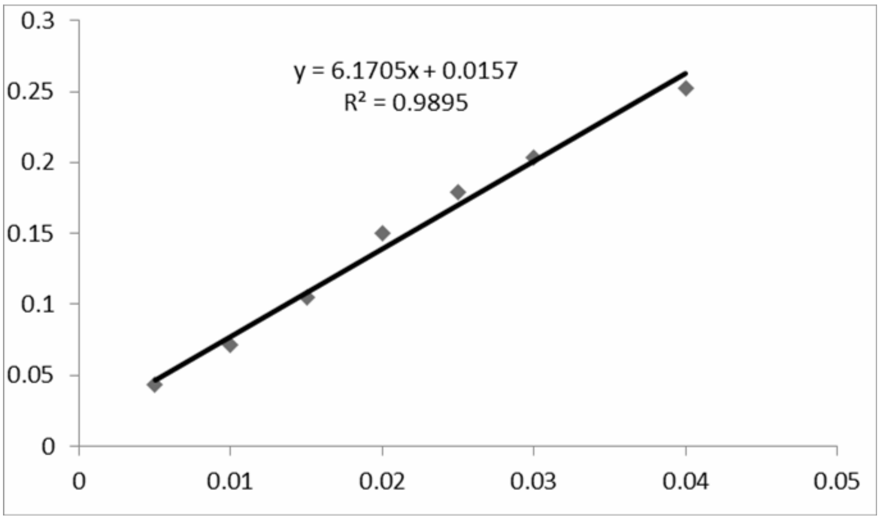

Fig. 2 – The calibration curve used to determine the nitrites concentration in fish meat and sediments.

Various physicochemical parameters were determined from the water samples, as follows: temperature, dissolved oxygen, pH, salinity, conductivity, chlorides and bicarbonates. For the determination of the parameters, the water auto analyzer Skalar San System and WTW multi-parameter 720 set were used.

The fish were caught using specialized fishing nets, authorized by ANPA (National Agency for Fisheries and Aquaculture) and the sediment samples were collected using WildCo instruments.

In case of RAS, fish were reared in 4 rearing units (B1, B2, B3, B4, 15 fish/unit), during a 54 days production cycle. The fish meat samples were prepared according to the STAS no. 11581-83 and after the diazotization reaction, the nitrite content was determined (Fig. 2) by using the spectro-photometer (AnaliticJena, Specord 210), at a wave length of 520 nm.

Also, the laboratory stage included preparation, storage and analysis of sediments samples, which were carried out according to M.E.S.P. (1987) and STAS 7184/2-77, STAS 7184/12-79 and STAS 7184/16-80.[22]

RESULTS AND DISCUSSION

The results from water analyses are presented in Table 1. It can be observed that data recorded for RAS registered the highest average values in terms of temperature (20.9°C), while the highest oxygen average concentration was recorded in DDB (9.85 mg/L), followed by MNP (9.66 mg/L), where it can be found the highest water salinity. The ponds (HLP and HLM) and RAS technological water registered the highest conductivity values, fact generated by the amount of daily administered fish feed, as they are considered aquaculture production systems. Therefore, as those three analyzed points are considered being intensive and semi-intensive aquaculture production systems, large amounts of fish feed are administrated and transformed into residual metabolites at their level. Those metabolites, together with the uneaten feed from the bottom of each fish rearing unit, enter into a decomposing process that induce high concentrations of nutrients into the water and determine the up-warding tendency of electrical conductivity, generating the significant higher values of this parameter in case of HLP, HLM and RAS. Another factor that can determine the significant high values of conductivity in case of the previous mentioned sample places is the high water temperature values, comparing with the ones registered at the rest of the sample places (Table 1). In case of DDB, DPT and DPG, it can be observed a high inverse proportional relation between the concentration of chloride and nitrites in water (Table 1 and Fig. 5), fact that can generate the highest nitrite toxicity, fact confirmed also by previous studies.[12] Also, the significant differences between MNP and MNG in terms of salinity, conductivity and Cl^- can be explain by the fact that MNG is situated at a confluence area between Danube River and the Black Sea and the water depth was considerably lower compared with MNP. It can be pointed out that lower levels of conductivity and salinity in case of MNG, compared with MNP, can indicate a high concentration of other major ions like K^+, Ca^{2+}, Mg^{2+} or SO_4^{2-}, compared with Cl^{-1} or Na^+.

Table 1

The water physico-chemical parameters, determined in each of the studied areas

		T°C	O_2	pH	SAL	µS/cm	Cl^- mg/L	HCO_3^- mg/L
MNP	AVG	15.05	9.66	8.27	4.5	488.17	473.06	247.48
	MIN	14.6	9.57	8.2	4.5	8.13	447.82	219.6
	MAX	15.5	10	8.4	4.5	8.21	500.28	292.8
MNG	AVG	15.91	7.08	8.15	0.2	547.28	68.24	233.54
	MIN	15.7	6.93	8	0.2	518	51.77	219.6
	MAX	16.1	7.57	8.4	0.2	572	80.01	268.4
DDB	AVG	16.02	9.73	8.12	0.15	496.14	44.61	257.94
	MIN	15.6	9.63	8	0.1	453	32.47	219.6
	MAX	16.4	9.85	8.2	0.2	541	59.97	292.8
HLP	AVG	16.64	9.59	8.81	0.8	1625.2	593.76	375.76
	MIN	16.4	9.55	8.8	0.8	1610	586.54	292.8
	MAX	17	9.64	8.9	0.8	1637	613.4	463.6
HLM	AVG	20.64	5.93	8.34	0.7	1485.57	475.98	247.48
	MIN	19.6	5.77	8.1	0.7	1479	471.78	195.2
	MAX	21.4	6	8.5	0.7	1490	488.23	292.8
DPT	AVG	17.94	7.14	7.97	0.18	603.42	52.33	306.74
	MIN	17.4	6.84	7.7	0.1	452	23.86	244
	MAX	19.6	7.28	8.3	0.6	1383	188.75	610
DPG	AVG	16.9	6.61	8.12	0.2	498.28	66.86	233.54
	MIN	16.5	6.58	8.1	0.2	483	59.54	195.2
	MAX	17.3	6.65	8.2	0.2	504	79.89	244
RAS	AVG	20.74	5.73	8.6	0.42	1040.57	204.32	103.78
	MIN	20.6	5.71	8.6	0.4	1007	201.73	83.46
	MAX	20.9	5.75	8.6	0.5	1140	206.96	124.11

Table 2

Feeding ration for each anthropogenic studied area

Anthropogenic sampling area	Feeding ratio
HLP	No feed was administrated
HLM	2% biomass weight/day
RAS	1% biomass weight/day

A number of 7 fish from each analyzed species were harvested from the anthropogenic environment, or cacthed from the natural environment. In case of the anthropogenic environment, the data related to feeding ratio are presented in Table 2.

Data related to analyzed fish biomass distribution are presented in Fig. 3 and Fig. 4. It can be mentioned that in case of RAS a normal distribution of fish individual biomass was observed at the beginning of the production cycle, while at the end of the experimental period, a heterogeneous distribution appeared (Fig. 3). This can be explained by the food competition manifested at the level of the fish population. The highest individual fish biomass was recorded in case of HLP – CRP and HLP – SMN (Fig. 4). In case of the natural environment, the biomass distribution of catches was heterogeneous (Fig. 4), fact influenced by the random fishing.

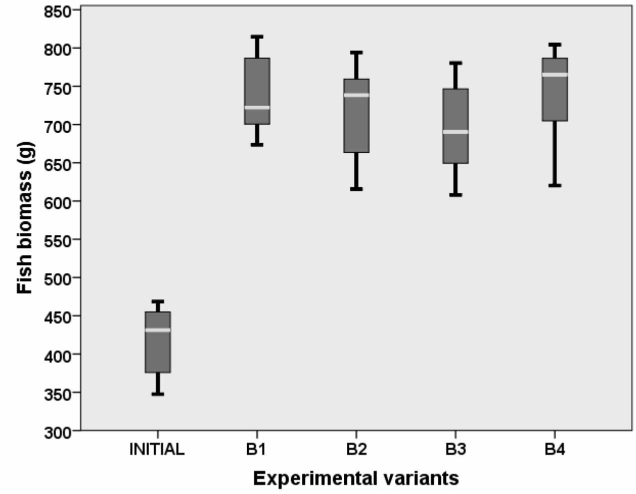

Fig. 3 – Fish initial and final (B1, B2, B3, B4) biomass distribution in RAS.

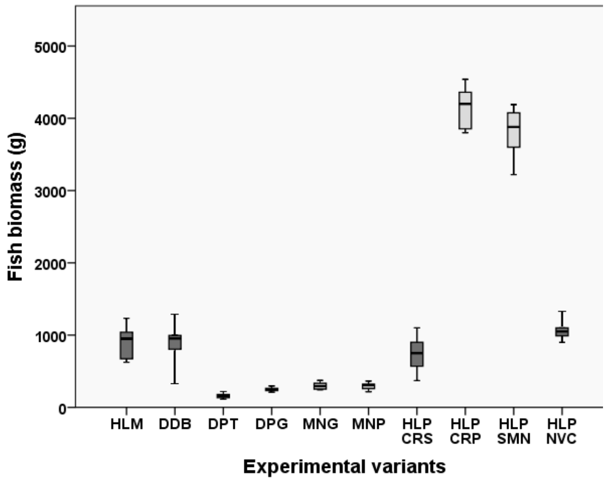

Fig. 4 – Fish distribution biomass in the natural and anthropogenic environment.

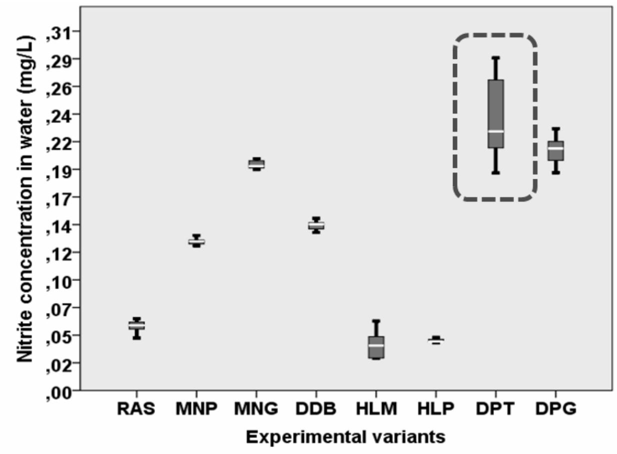

Fig. 5 – Distribution of nitrite concentration in water samples.

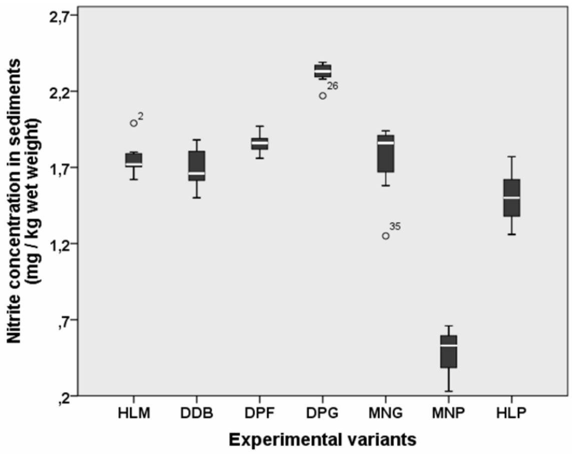

Fig. 6 – Distribution of nitrite concentration in sediments samples.

The nitrite concentration in water registered the highest values in case of DPT and DPG, while the lowest nitrite concentration was noted in the anthropogenic environment (Fig. 5). This fact can be justified by the presence of water treatment units integrated in the analyzed production systems (HLM, HLP and RAS). Within the biological filter of those production systems the occurring processes, described also by Granada et al. (2015), consists in the activity of two groups of bacteria that converts ammonium to nitrite and then to nitrate (NH_3 – NO_2 – NO_3), consuming a great deal of oxygen, lowering dissolved oxygen in the area. In Danube River harbor areas (DPT and DPG) the nitrite concentration in water registered a high variability (Fig. 5) because of the water currents and different wastewater outlet pipelines functioning regime.

The highest values of nitrite concentration in sediments was recorded in case of DPT and DPG (Fig. 6), fact directly and positively correlated with nitrites concentration in water, registered in those studied areas. The lowest concentration of nitrites in sediments were registered in the marine environment in MNP (Fig. 6), due to the existing anoxic conditions in the deep layer. In case of MNG, the results are higher (Fig. 6), although is considered marine environment, because the samples were taken from a confluence area between Danube River and the Black Sea and the water depth was considerably lower compared with MNP.

The concentration of nitrites in fish meat in case of RAS production systems had decreased during the time period of the production cycle (Fig. 7A). This fact is due to the presence of water conditioning units, presented in Fig. 1B, that are part of the RAS production system constructive configuration and have the purpose of maintaining the water quality between the optimum ranges for fish growth, especially in terms of ammonia, nitrites and nitrates concentration. The initial fish biomass used in RAS experiment derived from semi-intensive aquaculture systems, where the water conditioning units were poor, compared to RAS. Therefore, during the RAS production cycle, due to trickling biological filter intense ammonia and nitrites oxidation activities, a "purging process" occurred in terms of nitrites concentration in fish meat, fact that generates lower nitrite concentration at the end of the production cycle, comparing with those from its beginning. The results obtained for nitrite concentration in fish meat at the end of RAS experiment, were relatively similar with those obtained by Petrea et al. (2014), that registered a nitrite concentration in stellate sturgeon (*Acipenser stelatus*) reared in RAS conditions between 0.18 and 0.41 mg/kg fresh weight. Also, Petrea (2014) had found nitrite concentration in rainbow trout meat, reared in RAS conditions, between 0.16-0.22 mg/kg fresh weight.[10-11]

The highest nitrite concentration in fish meat, in the natural environment, was found in case of MNG, fact explained by the sampling point position. Therefore, the sampling point is encountered exactly at the confluence of Danube, which is known to be the principal pollutant of the Black Sea, with the Black Sea (Fig.7B).

In case of HLP, it can be observed a small distribution range of nitrite concentration in fish meat, most probably because all fish are constantly exposed to the same water physico-chemical parameters, as they are reared in a pond aquaculture system and the lateral swimming space is limited (Fig. 7B). The wide distribution range of nitrite concentration in fish meat found in the case study area from both Danube and Black Sea (DDB, DPT, DPG, MNG and MNP), are due to the migratory behavior of fish. Therefore, in the migration process some fish might reach polluted "hot spots" areas and remain there for a longer period, as the feeding conditions are better. Therefore, this can strongly influence the nitrite concentration of their muscle tissues. In case of HLM, the wide distribution range (Fig. 7B) can be explained by the fact that the fish originate from different fish farms and they were reared in different conditions. Therefore, as it is known to be a direct correlation between the time exposure to a pollutant (in this case nitrites) and the concentration of this pollutant in fish tissues, it can be mentioned that aquatic environment conditions from HLM did not have the necessary timeframe to influence, in a significant way, the concentration of nitrites in fish meat, moreover the fish were influenced by the water chemistry from the origin aquaculture systems.

It can be observed a strong positive linear correlation (Fig. 8A and Fig. 8B) between fish individual biomass and the nitrite concentration in fish meat, in case of all studied areas (anthropogenic and natural environment). However, no significant correlations were found between nitrite concentrations in sediments, water and fish meat.

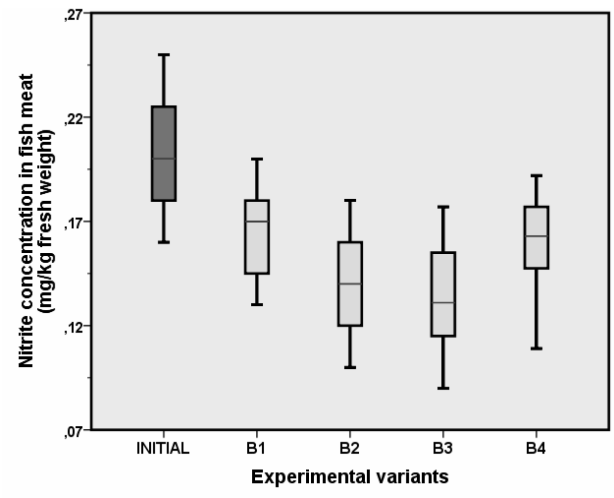

Fig. 7 A – Distribution of nitrite concentration in fish meat, in RAS experimental variant.

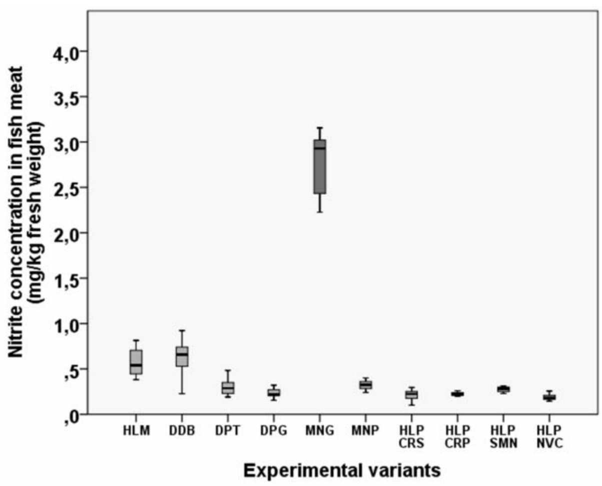

Fig. 7 B – Distribution of nitrite concentration in fish meat in both natural and anthropogenic environment.

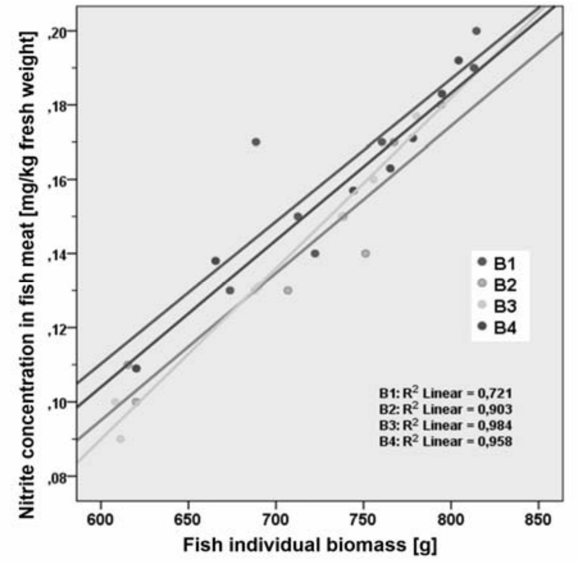

Fig. 8 A – The linear regression between nitrite concentration in fish meat and fish biomass in RAS.

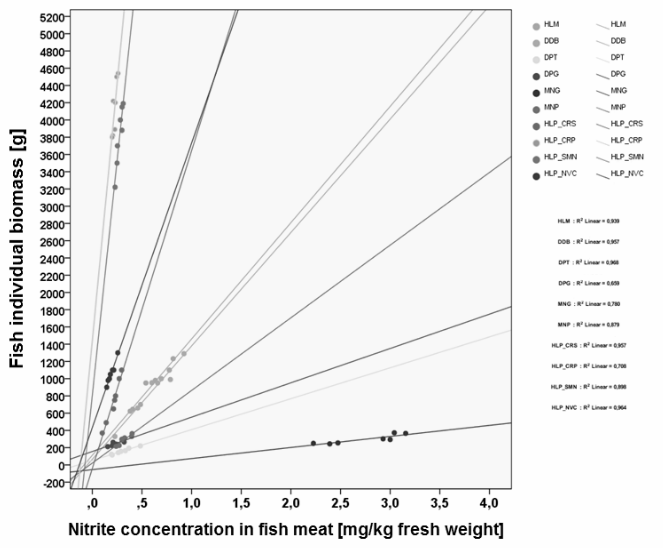

Fig. 8 B – The linear regression between nitrite concentration in fish meat and fish biomass in both natural and anthropogenic environment.

CONCLUSIONS

As a main conclusion to this study, it can be pointed out that there is a strong positive correlation between fish biomass and the concentration of nitrite in fish meat, both in anthropogenic (RAS and ponds) and natural environment. Also, RAS can be a solution in order to decrease the nitrite concentration in fish meat, if the optimum water quality parameters in terms of nitrite concentration are within the optimum range for a specific fish species.

No correlations were found between nitrite concentration in water, sediments and fish meat in the natural environment.

It is recommended that future research should include other analysis (like redox potential) in order to correlate the nitrite concentration from water, sediments and fish.

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