

## WARM-WATER FISH FARMING AFTER SOIL REMEDIATION

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### Abstract

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On land where once stood a slaughterhouse landfill, remediation of soil was performed by removing 1m of soil and thereby removing organic material. After that, a fish pond construction was done and the land which was used for that purposes was brought from a site outside of the landfill. Prior to starting the fish production, agricultural limestone was applied to the bottom of the empty pond in an amount of 2000 kg/ha. The fish pond was subsequently filled with water from wells in the depth range of 75 to 95 m, and the quality of the water was equal to the quality of second class water in accordance with the regulations of the Republic of Serbia. One-and two-year old common carp were stocked in the fish pond and the total stocking density was 600 kg/ha. During the fish farming the formulated feed with 32% protein and 10% fat was used and besides that a by-products form slaughterhouse (spleen, liver, lung) supplemented with grains. Aeration was provided by the spraying of the well water and by an aerator that pulled the water from the water layers close to bottom of fish pond. The flow of the water was 10 s L/ha. During the production season continuous monitoring of basic environmental conditions, particularly the amount of dissolved oxygen, biological oxygen demand, chemical oxygen demand, quantification of consumption of permanganate, amount of nitrite, nitrate, chloride, total phosphorus, ammonium ions and ammonia and pH was done. Additionally the production parameters and health of the fish were observed. Harvesting density was 5000 kg/ha.

**Key words:** soil remediation, carp production, ecology, environmental conditions

**Abbreviations:** COD – chemical oxygen demand; BOD – biological oxygen demand; ISO – international organisation for standardisation; SRPS – serbian organisation for standardisation; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; USFA – unsaturated fatty acids; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid

### Introduction

Until now there has not been a lot of data in the literature relating to the construction of a pond on the land which served as a landfill and where remediation was performed. This is one of the first papers dealing with this issue. In the era of worldwide increasing demands for sustainable usage of primary resources, such as land and water, this type of land ex-

ploitation should be considered. This production also affects rural development and environmental protection (Ljubojević et al., 2011). One of the first things that must be done is to remove the surface layer of soil to reduce the amount of organic matter (Pelić, 2014). Location for fish pond construction is a very important and sometimes has crucial importance for the financial success of companies (Ćirković et al., 2015). It is particularly important that the water used for the pond has

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optimal physical and chemical characteristics for fish breeding and that there is enough water during the whole growing season (Ćirković et al., 2015). Zooplankton and phytoplankton are also very valuable indicators of water quality (Attayde and Bozelli, 1998; Hakkari, 1972; Pontin and Langley, 1993; Zakaria et al., 2007; Wu, 1984; Weber and Weber 1998). For successful carp production it is necessary to provide adequate environmental conditions, density of population, balanced diet and adequate handling with fish (Ćirković et al., 2012). Serbia is a country with traditional production of common carp, and as such occupies third place in the production of carp per capita in the world, behind the Czech Republic and China, with a production of 1.3 kg per capita (Ljubojević et al., 2014). Fish meat and fish products represent a valuable source of nutrients of great importance for a diverse and healthy diet. Proteins from fish meat are characterized by favorable amino acid composition with a lot of free amino acids (Buchtová et al., 2010) and contain all the essential amino acids the human body and may be the only source of animal protein in the diet (Vladau et al., 2008). Fish meat contains high omega-3 polyunsaturated fatty acids content such as eicosapentenoic acid (20:5 n-3, EPA) and docosahexaenoic (22:6 n-3, DHA) (Ljubojević et al., 2013). Adequate health care and appropriate intervention aims to preserve and improve fish production (Ćirković and Novakov, 2013).

The aim of this paper is to show that it is possible to produce fish on land where remediation has been done, which is a significant contribution to environmental protection and rural development.

## **Material and Methods**

The experiment was conducted within the meat industry "Agropapuk" in Kukujevci. The land where pond was built was used as a village landfill. Construction of the fish pond started by removing a soil layer with a thickness of 1 m. 12000 m<sup>3</sup> of excavated land was transported and disposed of, 12000 m<sup>3</sup> was dug up and used, and 6000 m<sup>3</sup> was brought from the site outside of the landfill. Production took place in an area under the water of 5 ha.

Before starting the fish production, agricultural limestone was applied to the bottom of empty pond in an amount of 2000 kg/ha. Filling of the fish pond with water was completed from two wells with a depth range of 75 to 95 m, using a pumps capacity of 1000 l/min for each well. Aeration was conducted using aerators (mud pumps) with a capacity of 2000 l/min spraying the water 5 m above the surface. The flow of the water was 10 s L/ha.

Production of carp was carried out during 2014 growing season from 15 of April to 20 of October. One-and two-year

old common carp were stocked in the fish pond and the total stocking density was 600 kg/ha, with a ratio of 400 kg/ha for two-year old and 200 kg/ha for one-year old carp. The average weight of an one year old carp was 60 g and a two-year old carp was 600 g.

During the production, commercial extruded and pelleted fish feed, with 32% of proteins and 10% of fat, were given to fish. Besides that, food obtained in a slaughterhouse as by-products of slaughterhouse industry (spleen, liver, lungs) with the addition of cereals (20% of soybean meal, 10% of sunflower meal (44% protein), 2%, of yeast, wheat and maize) was added. This mixture contains a total of 45% protein and 9% fat in dry matter. Feeding was carried out twice a day in 8 am, and the 3 pm.

The flow of the water was 10 sec L/ha. During the production season continuous monitoring of the basic environmental conditions was undertaken twice a week, particularly the amount of dissolved oxygen, biological oxygen demand, chemical oxygen demand, quantification of consumption of permanganate, amount of nitrite, nitrate, chloride, total phosphorus, ammonium ions and ammonia and pH.

The use of hydrated lime was 1000 kg/ha in April, May and June, while in July, August, September and October was 2000 kg/ha.

During the growing season the health status and condition of the carp was regularly controlled at least twice a month.

Quality control of the pond water was tested with standard methods (SRPS, SRPS EN ISO, EPA). The pH of a solution is measured as negative logarithm of hydrogen ion concentration. At a given temperature, the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration.

The Chemical Oxygen Demand (COD) test determines the oxygen requirements equivalent of organic matter that is susceptible to oxidation with the help of a strong chemical oxidant. COD is determinate by titration method by using dichromate.

The Biochemical Oxygen Demand (BOD) is an empirical standardized laboratory test which measures oxygen requirements for aerobic oxidation of decomposable organic matter and certain inorganic materials in water, polluted waters and wastewater under controlled conditions of temperature and incubation period. The quantity of oxygen required for the above oxidation processes is a measure of the test.

KMnO<sub>4</sub> indicates organic pollution. It is determinate by titration in the acid medium.

Dissolved Oxygen – All living organisms are dependent upon oxygen in one form or another to maintain the metabolic processes that produce energy for growth and reproduction. Dissolved oxygen was measured by portable meter WTW OXY 300.

Ammonia is produced by the microbiological degradation of organic nitrogenous matter. Ammonia produces a yellow coloured compound when it reacts with alkaline Nessler reagent, provided the sample is clarified properly. Ammonia is determined by measuring absorbance with UV/VIS spectrophotometer.

**Nitrogen (Nitrate) ( $\text{NO}_3^-$ )** Nitrate is the most highly oxidized form of nitrogen compounds commonly present in natural waters. Significant sources of nitrate are chemical fertilizers, decayed vegetable and animal matter, domestic effluents, sewage sludge disposal to land, industrial discharge, leachates from refuse dumps and atmospheric washout. Nitrates are determined by measuring absorbance with UV/VIS spectrophotometer.

**Nitrogen (Nitrite) ( $\text{NO}_2^-$ )** Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. As an intermediate stage in the nitrogen cycle, it is unstable. Nitrite was determined by measuring absorbance with UV/VIS spectrophotometer.

**Chloride ( $\text{Cl}^-$ )** The presence of chloride in natural waters can be attributed to dissolution of salt deposits or discharges of effluents from chemical industries. They are determined by titration.

**Phosphate and orthophosphate** are determined by spectrophotometer measurement.

**Table 1**

**Quality of well water and pond water before and after addition of lime, the range of maximum and minimum concentration of tested parameters**

The tested parameter	Unit	Method	The measured value (before addition of lime)	The measured value (after addition of lime)	The measured value (well water)
pH		SRPS H.Z1.III: 1987	7.4–7.9	7.8–8.4	7.5–7.7
Chemical Oxygen Demand (COD)	mgO <sub>2</sub> /l	SRPS ISO 6060:1994*	20–26	5–10	2.83–3.81
Biochemical Oxygen Demand (BOD)	mg O <sub>2</sub> /l	H1.002*	6–9	< 4	< 4
KMnO <sub>4</sub>	mg/l	SRPS EN ISO 8467:2007*	30.8–35.6	21–26	1.53–1.80
Dissolved Oxygen	mg/l	SRPS ISO 5814:1994	5.8–6.2	7.56–7.94	5.0–5.6
Total N	mgN/l	Computing	2.20–2.90	1.21–1.72	2.07–2.71
Ammonia	mgN/l	SRPS ISO H.Z1.184:1974*	0.7–1.50	0.42–0.522	<0.02
Nitrate	mgN/l	SRPS ISO 7890-3:1994*	0.37–0.42	0.43–0.46	1.60–1.82
Nitrite	mgN/l	SRPS EN 26777:2009*	0.04–0.060	0.03–0.057	0.003
Chloride	mg/l	SRPS ISO 9297:1997 SRPS ISO 9297-1:2007	20–26	18–22	8–10
Phosphate	mgP/l	SRPS EN ISO 6878:2008*	0.035–0.042	0.011–0.013	0.026–0.031
Ortophosphate	mgP/l	SRPS EN ISO 6878:2008*	0.021–0.029	0.006–0.012	0.015–0.021
Metals					
Iron	mg/l	EPA 7000b*	< 0.068	< 0.068	< 0.068
Zink	mg/l	EPA 7000b*	< 0.011	< 0.011	0.021–0.025
Arsenic	µg/l	EPA 7010*	2.06–2.75	< 1.37	< 1.37

**Metals** – all metals are determined by AAS.

**Chemical analysis.** Water content of fish fillets was determined after drying the samples at 105°C to a constant weight for 24 hours (SRPS ISO 1442:1997). Crude protein content was determined by Kjeldahl and ash was determined after burning. Crude lipid in fish tissue was also analyzed using the Soxhlet System with ether as a solvent (SRPS ISO 1443:1997).

Extraction of lipids was done using Spirić et al. (2010) method for extraction of lipids from fish muscle by ASE.

Fatty acid Methyl esters were determined by Gas Chromatography.

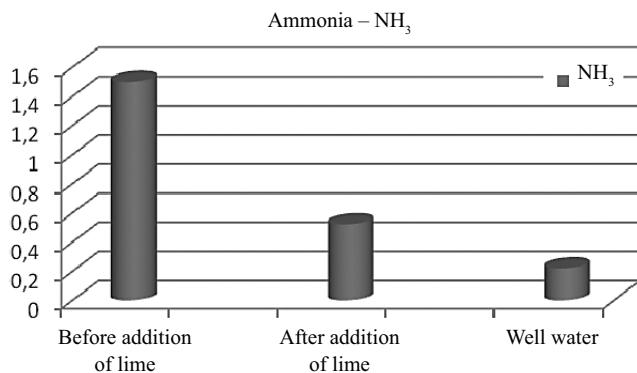
## Results

At the end of the growing season, the average weight of the two-year old carp was 900 g, and 3.8 kg for a three-year old carp. Harvesting density was about 5000 kg/ha.

Conversion was 0.9 for formulated feed and 0.8 for additional mixtures counting in the dry matter. Mortality ranged from 5% in two-year old carps, and 10% in one-year old carps.

Table 1 shows the range of the maximum and minimum concentration of tested parameters in well water and the water from the fish pond before and after the addition of lime.

The Results of the analysis of the well water indicate that it has a very good quality which corresponds to the quality of drinking water. In the pond water the highest values reach level of ammonia, which decreases after using lime and descends into the acceptable values as shown in Figure 1.



**Fig. 1. Level of ammonia in well water and in the pond water before and after addition of lime**

Table 2 summarizes the chemical content and fatty acid composition of the sampled carps. It should be noted that 12 specimens were taken for analysis. The amount of protein was 16.21 % and fat content 4.42%. A lipid analysis enabled the classification and quantitative determination of fatty acids and besides that the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 acids, n-6 acids, n-3/n-6 ratio, n-6/n-3 ratio and ratio of PUFA/SFA, as well as the ratio of USFA/SFA which represent the indicators of lipid quality. The total amount of SFA was (27%), the amount of MUFA was (28%) and PUFA (44%) content was the highest. The ratio of n-3/n-6 was 0.92, while the ratio PUFA/SFA was 1.62.

During the health status of fish monitoring erythrodermatitis was diagnosed. The disease appeared from the middle of August with prevalence of 25%. After treatment with flumequin in pelleted feed, the symptoms of disease disappeared.

## Discussion

The results of the growth indicate that it is possible to breed carps in ponds where remediation has been performed and where there is a lot of organic material, which could not be completely eliminate by removing the surface layers of soil. The favorable weight of the carp at harvesting is the result of good environmental conditions that were based on the use of well water, adequate flow, aeration, use of hydrated lime, quality of complete feed and feed supplements from the slaughter industry.

**Table 2**

**Chemical and fatty acid composition of produced carp**

Parameter	Carp
Water content (%)	78.36 ± 0.24
Protein content (%)	16.21 ± 0.12
Fat content (%)	4.42 ± 0.17
Ash content (%)	1.02 ± 0.02
Fatty acid, %	
C12:0	0.06 ± 0.01
C14:0	1.53 ± 0.25
C15:0	1.11 ± 0.08
C16:0	18.35 ± 0.28
C17:0	1.32 ± 0.09
C18:0	4.51 ± 0.12
C20:0	0.26 ± 0.03
SFA	27.15 ± 0.38
C16:1, n-9	5.73 ± 0.28
C18:1cis-9, n-9	19.39 ± 0.21
C18:1cis-11, n-7	2.33 ± 0.27
C20:1, n-9	1.35 ± 0.12
MUFA	28.79 ± 0.48
C18:2, n-6	10.29 ± 0.11
C18:3, n-6	5.22 ± 0.06
C18:3, n-3	5.96 ± 0.14
C20:2, n-6	0.33 ± 0.07
C20:3, n-6	0.91 ± 0.06
C20:3, n-3	0.89 ± 0.05
C20:4, n-6	6.21 ± 0.11
C20:5, n-3	4.05 ± 0.08
C22:5, n-3	4.47 ± 0.24
C22:6, n-3	5.75 ± 0.48
PUFA	44.08 ± 0.55
n-6	22.96 ± 0.20
n-3	21.12 ± 0.48
n-3/n-6	0.92 ± 0.02
n-6/n-3	1.09 ± 0.02
PUFA/SFA	1.62 ± 0.04
USFA/SFA	2.68 ± 0.05
PUFA/MUFA	1.53 ± 0.04

(Data are means ± S.E.M. (n = 12). Different superscripts within the same rows differ (P < 0.01), USFA – unsaturated fatty acids, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids)

The accumulation of contaminants in the fish tissues depends on their concentration in the water, the lifetime of the fish, the age and the amount of body fat of fish (Bordajandi et al., 2006). Direct transfer of pollutants is most common from the environment in the sediment and from the sediment

into the aquatic environment and aquatic organisms. State of the ecosystem, water and sediment directly affects the quality and safety of fish meat (Zoumis et al., 2001). There is a possibility of mobilization of pollutants in meat and organs of fish (Balter and Lecuyer, 2010), which in the pond can reach as a result of pollution of the immediate surroundings. The degree of contamination of water and harvested fish from an ecosystem can indirectly serve as a bioindicator of the degree of contamination of the ecosystem (Jankovic et al., 2011). Despite the removed soil, organic production was high as well as the amount of ammonium, which was balanced with the constantly adding of hydrated lime (the amount which is higher than usual). This helped to keep environmental conditions in the optimum level for the cultivation of fish, so we had no problem with the smaller intake of food, lack of oxygen level and higher mortality of fish. The results of the analysis of the water from fish pond explain the reasons for it being built on remediate soil.

In recent years, increased attention has been paid to the fact that the ponds create a large amount of nitrogen, phosphorus and organic matter in the water. Nutrients which may cause eutrophication of the water coming either directly from the feed or fish excrement (Watanabe et al., 1999). The easiest way to reduce the intake of these substances in the water, is usage of sufficiently stable feed in the water which contains nutritional components that fish can use maximum (Cho and Bureau, 2001; Radosavljevic et al., 2014). All these facts are taken into account during the breeding of fish, so the diet was carried out using a complete feed for carp. In addition, it was made and the feed was a mix of grains and internal organs of animals from slaughterhouses. The results indicate that this type of diet achieved more than good production results and the amount of pollutants in the analyzed water was minimal. By analyzing the economic parameters it can be seen that they are favorable due to good conversion. It is necessary to notice that the pond is the property of the slaughterhouse industry and the additional food from slaughterhouses actually is not actually added in the cost of a kg kilogram of fish.

The meat quality of carp fish is very variable and changes under the influence of age, breeding systems and nutrition. The fat content in the carp generally ranges from 2.3 to 16.8%, while the protein content is less variable and generally is in the range of 14 to 18% (Vladau et al., 2008; Trbović et al., 2009; Ćirkovic et al., 2011). Protein and fat content (16.2; 4.4) of analyzed carp move within the following limits and is very favorable. The results of SFA and USFA obtained in this study are comparable with the results of other authors. The ratio of n-3/n-6 ranged from 0.8 to 2.4 according to Steffens and Wirth (2007). The ratio of PUFA/SFA which is an indicator of the quality of lipids was also beneficial (1.62). As the favorable

fatty acid composition of carp affected the entire mixture and mix of grains and liver, spleen and lung, it has been found that the complete mixture has a very favorable effect on the quality of the fish meat (Ljubojević et al., 2013).

Fish eating birds were not present in the pond, and therefore have not been taken into account with regards to the fish mortality.

## Conclusions

It is possible to produce fish on land where remediation has been conducted. Continuously monitoring of the environmental conditions during the entire process of production is necessary.

Using well water has an advantage over the water from open water courses, due to its favorable chemical composition. Water flow and aeration are required in a system with a higher organic load of water. The use of hydrated lime is a limiting factor in fishery production in organic loaded water.

High production is the result of good nutrition and good environmental conditions. Favorable conversion per kg was the result of quality balanced meals.

The results of chemical composition of carp meat shows that a very high-quality food is produced.

In monitoring the fish's health status and adequate therapeutic measures, implementation is very important in intensive production, especially in the ponds where a high level of organic matter is expected.

Remediation contributes to environmental protection and rational usage of land area.

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