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IMPACT OF FISH POND MANURING ON MICROBIAL WATER QUALITY

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Abstract

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Fish pond manuring is often used in fish farming for intensification of fish production by balancing the ratio between carbon and other nutrients. However, the using of manure, classified as hazardous organic matter originating from animal faeces, poses a risk to the water environment.
The aim of this study was to evaluate the effect of semi-liquid pig manure application on faecal pollution and related potential health risk of fish pond utilization.
The evaluation of faecal pollution was made during 6 years; the microbiological monitoring was completed with the water chemistry data.
Our results showed that a better situation was in ponds, processes of which can be regulated by changes in nutrient contents, i.e., by controlled manure application. The absolute counts of microbial indicators were not significantly different in the control non-manured and manured fish ponds, but sporadically detected high faecal indicators counts in summer can represent a potential health risk.
According to our results, the water quality of fish ponds is influenced due to manuring but the immediate impact of manuring in fish ponds and recipients in our study proved neither in deterioration of microbiological quality and health risk enhancement nor in the water quality assessed by chemical analyses.

microbial faecal pollution, fish ponds, manuring, water quality

The Czech Republic is one of several countries in the world where fish farming has a long tradition. At present, there are about 24 000 ponds with surface area approximately 52 000 ha that are used for fish farming and about 20 000 tons of market-size fish (carp represents 85%) are annually produced. Fertilizing of ponds in the Czech Republic is subjected to the water authority permission.

Organic manuring represents important means of fish farming production intensification, in consequence of balancing the proportion of carbon to the content of other biogenic elements (especially nitrogen and phosphorus). Increased carbon content results in providing a sufficient amount of nutrients for phytoplankton, which in conjunction with supply of microbial inoculum responsible for mineralization of organic matter, serves as a food source for zooplankton which is important for natural fish food (Dhawan and Kaur, 1978; Adámek and Jirásek, 1989). Different kinds of manure can be utilized; cow and poultry dung and semi-liquid pig manure are of the highest interest (Govind *et al.*, 1978; Wohlfarth and Schroeder, 1979). All kinds of livestock manure are classified as hazardous substances; their application may be associated with the deterioration of water quality in fish ponds and surface water recipients. Excessive use of manures may present a risk related to the creation of unbalanced conditions in water ecosystem, such as oversupply of nutrients, eutrophication, decay of organic matter and presence of enteric pathogens. Microbiological analyses revealed presence of various pathogenic microorganisms in manure in addition to the common microflora of animal intestine. The occurrence of *Escherichia coli* O157, *Salmonella*, *Yersinia enterocolitica*, *Campylobacter*, *Listeria monocytogenes* etc. is described most often (Quines, 1988; Pell, 1997; Guan and Holley, 2003). Persistence of pathogens in manure and water environment is considered as one of the crucial factors for infection

transmission. It was found that zoonotic pathogens can survive in such environments up to 4 months, depending on the type of manure, temperature, pH, oxygen level, ammonia concentration and presence of competing organisms (Jones, 1976; Guan and Holley, 2003). Nevertheless, it was proved that elimination of harmful effects of substances from the manure and incorporation of nutrients into the productivity chain is enabled in functional and balanced ecosystem of fish ponds (Adámek and Jirásek, 1989).

The aim of this study was to evaluate the microbial water quality changes by using semi-liquid pig manure for fish production intensification and define related potential increase of health risk.

MATERIALS AND METHODS

Studied localities

11 fish ponds in the area of the Czech-Moravian Highlands in the Czech Republic were studied during years 2004–2009. The studied ponds were with different treatment. Five of them were used for fish production and in all monitored years fertilized by manuring. Three ponds had more influences and pollution sources: one from manuring and municipal pollution from waste water treatment plant and effluent disposal; two from manuring and waterfowl breeding. Three ponds were chosen as "control" sites, i.e. were without any water-use and significant water pollution sources. Manuring of the ponds was carried out once a year in spring with manure dose from 50 to 200t dependent on the cubic content of pond and presence of other nutrient sources. The manure was applied on one place usually near the feed object. The manure application in Czech Republic is subject to a decision of a national regulatory authority for application of hazardous substances.

Sampling

Monitoring of faecal pollution of all selected fish ponds was carried out during 5 years (2004–2009). In total, 140 grab water samples were collected in weekly to monthly intervals from the surface of fish ponds before and after manuring which was usually performed in spring months (45 samples from control non-manured ponds, 10 samples from ponds before manuring, 60 samples from ponds after manuring, 10 samples from municipal polluted ponds after manuring, 10 samples from municipal polluted ponds without manuring, 5 samples from ponds with waterfowl breeding, after manuring). The sampling localities were situated near the dam of the fish pond. For fertilizing, semi-liquid pig manure was used in all cases. Altogether, 12 sediment samples were taken before and after manuring and were pooled from 5 sampling sites from fish pond. The survey in 2007 was extended by water pollution monitoring of fish pond inflows and outflows. Samples of semi-liquid pig manure used for the treatment of fish ponds were analysed for survival of faecal bacteria.

Sample analyses

Microbial water quality was in all samples described by indicator microorganisms detection, including faecal coliform bacteria (FC), *Escherichia coli* (EC), enterococci (ENT), heterotrophic plate counts at 22°C (HPC). In selected samples with higher probability of pathogenic bacteria presence, i.e. in manure, fresh manured ponds and high summer season, *Salmonella* and *Escherichia coli* O157 were detected.

Water samples were characterized also by the basic physical-chemical and chemical parameters. However, these results in this study were used as supplementary for microbial data and understanding of processes. The microbial quality of water samples was evaluated according to the limits of Czech Government Decree on bathing water (i.e. seasonal mean value < 300 and < 100 CFU (colony forming units).100 ml^{-1} *E. coli* and enterococci, respectively; in this study the data compared to the limits were individual values and not mean values as required by the Czech Government Decree). Sediment and semi-liquid pig manure samples were subjected only to microbiological analyses.

Detection of faecal indicator bacteria, organic indicator bacteria and *Salmonella* in water and semi-liquid manure samples

For indication of faecal pollution, bacterial faecal indicators were detected. Faecal coliform bacteria and *E. coli* were determined according to the National Standards for detection and enumeration of thermotolerant coliform bacteria and *Escherichia coli* based on cultivation on m-FC agar at 44 °C and detection of β-D-galactosidase-positive colonies (ČSN 75 7835). Assay of β-D-glucuronidase activity was used to detect *E. coli* among β-D-galactosidase-positive colonies. For the determination of enterococci, membrane filtration method according to the EU Standards for detection and enumeration of intestinal enterococci was used (ČSN EN ISO 7899-2). For the determination of heterotrophic plate counts at 22 °C, the procedure described in the EU Standards for enumeration of culturable micro-organisms was used (ČSN EN ISO 6222). This parameter serves for indication of easily utilizable organic matter presence. Potential health risk represented by pathogenic bacteria contamination was proved by detection of *Salmonella* and *E. coli* O157. *Salmonella* was detected according to the Czech National Standard for detection of presence of bacteria from the genus *Salmonella* (TNV 75 7855). This method is based on non-selective enrichment followed by selective enrichment and cultivation on selective agar media. Biochemical tests are used for confirmation of suspected isolates. *E. coli* O157 was detected by the internal procedure using selective multiplication in mTSB broth with novobiocine

and selective cultivation on MacConkey agar with sorbitol.

Detection of faecal indicator bacteria and *Salmonella* in sediments

Faecal indicators in sediments were determined using internal procedure as follows. Sediments were mixed in the bottle, and then 100 g of the sample was transferred into a plastic bag and homogenized in the homogenizer for 10 s. 40 g of the sample was diluted with 60 ml of physiological saline and shaken on the horizontal shaker for 30 min. After 5 min of sediment settling, 1 ml of supernatant was inoculated on m-FC agar for the detection of faecal coliform bacteria and *E. coli* or on Slanetz-Bartley agar for the determination of enterococci according to standard methods (ČSN 75 7835; ČSN EN ISO 7899-2). *Salmonella* in sediments was detected according to the EU Standard for sludge characterization (CEN/TR 15215-3).

Testing of faecal indicator bacteria survival in semi-liquid pig manure

Survival of faecal indicator bacteria in semi-liquid pig manure stored at dark at 6 ± 2°C in a closed plastic bottle was monitored for more than two years. Samples were at first taken quarterly, then in a longer period and analyzed for the presence of faecal coliform bacteria and enterococci.

Data evaluation

In this study analytic data from 11 fish ponds (3 control, non-manured; 5 manured and 3 with combined effects, i.e. manuring together with municipal pollution or with waterfowl breeding) were evaluated. The evaluation was made by comparison of following sets of results for faecal (FC, ENT and EC – "FAECAL") and organic (HPC – "ORGANIC") parameters (labelling used in Figures is in quotation marks):

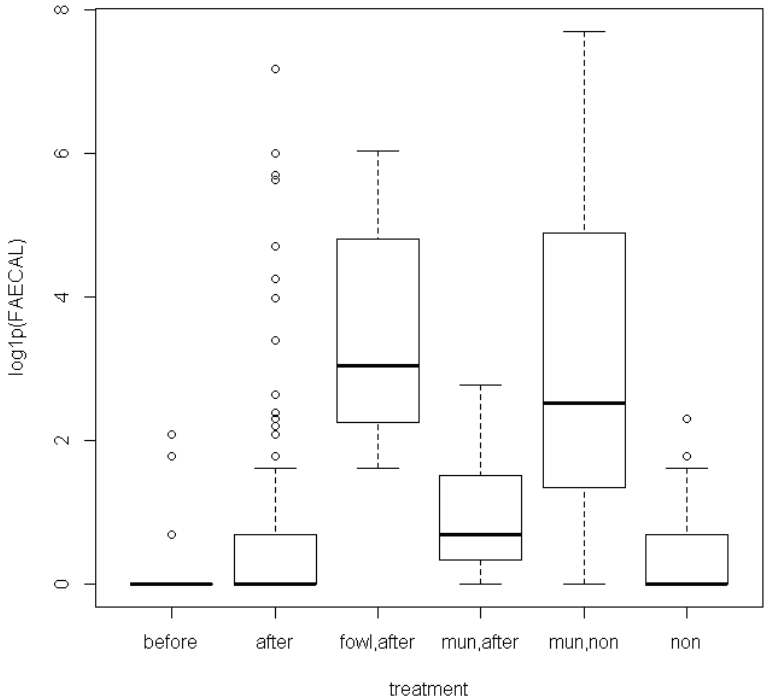
- ponds before manuring ("before"),
- ponds after manuring ("after"),
- ponds with waterfowl breeding after manuring ("fowl, after"),
- ponds with municipal pollution after manuring ("mun, after"),
- non-manured ponds with municipal pollution ("mun, non") and
- non-manured ponds ("non").

The data are presented after processing with software R, version 2.11.1 by comparison of box plots with interquartile range, median, minimum and maximum for each evaluated data set (Fig. 1, 2).

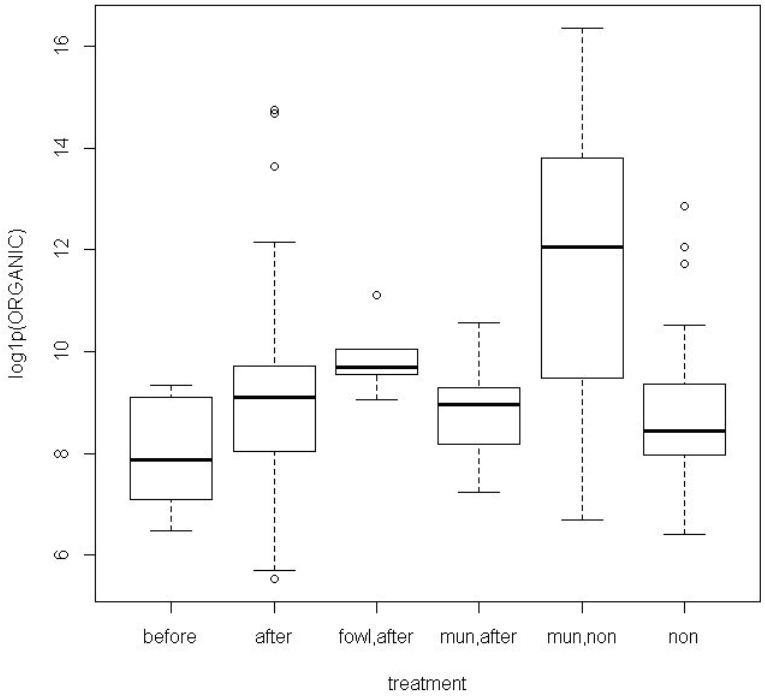
RESULTS AND DISCUSSION

Water quality in fish ponds

Our results proved unexpectedly low microbial counts in all of manured as well as of control non-manured fish ponds. Microbial contents in fish pond water samples corresponded to non-contaminated surface water samples (Wohlfarth and Schroeder, 1979). Counts of faecal indicator bacteria in non-manured fish ponds (labelled as "before" and "non") reached the values of 0 to 900 CFU.100 ml^{-1}. A content



1: *Occurrence of faecal indicator bacteria in studied fish ponds. Note: For abbreviations see Material and Methods, Data evaluation.*



2: *Occurrence of organic indicator bacteria (heterotrophic plate counts-HPC) in studied fish ponds. Note: For abbreviations see Material and Methods, Data evaluation.*

of biodegradable organic matter and assimilable organic carbon was derived from heterotrophic bacteria amounts. Numbers of heterotrophic bacteria in comparison to manured ponds were low; mostly up to 10^3 CFU.1 ml^{-1}. Analyses of chemical parameters showed specific character of pond water, independently on ponds utilization. The ponds water had relatively low conductivity and ammonia content, despite the concentration of organic substances and phosphorus indicated high water pollution. Dissolved oxygen content and pH values varied depending on water temperature and algae abundance (determined by measurement of water transparency).

Studied manured fish ponds (labelled as "after") contained low numbers of faecal bacteria (Fig. 1), similarly to the non-manured fish ponds (labelled as "non"). The counts of faecal indicator (FC, EC, ENT) were mainly 0 to 1 000 CFU in 100 ml, organic pollution indicators (HPC) were usually from 10^2 to 10^5 CFU in 1 ml (Fig. 2). However, high amounts (to 40 000 CFU.100 ml^{-1}) of faecal bacteria (FC, EC, ENT) were sporadically found in most of the manured ponds in late summer. The strict values of Czech Government Decree for bathing waters in the evaluated period were mostly not achieved already in ponds before manuring (30% cases) and in more then 50% individual values in manured ponds. Mentioned high numbers of faecal indicators (Fig. 1, labelled as "after") could represent a potential health risk in case of recreational use of fish ponds. The heterotrophic bacteria counts increased after the input of large amount of light biodegradable organic matter and bacterial inoculum in spring by manure application (Fig. 2, labelled as "after"). This phenomenon is the basis of properly functioning cycle of nutrients, microorganisms, plankton and fish in fish ponds.

The negative impact of manuring was proved immediately neither in microbiological quality nor in water quality assessed by chemical analyses. The explicit connection of occurrence of high numbers of faecal indicators, i.e. increasing of health risk by manure application, was not proved in this study. It was also confirmed by negative findings of pathogens in fish pond water samples.

As the most problematic were evaluated the fish ponds impacted by other influence, than manuring (labelled as "mun, non") or by more types of contamination, i.e., combination of manuring and municipal pollution (labelled as "mun, after") or waterfowl breeding (labelled as "fowl, after"). The numbers of faecal and organic indicators were an order of magnitude higher than in manured fish ponds (Fig. 1, 2). The values in the evaluated period exceeded the Czech Government Decree on bathing waters for microbial parameters in more than 80% of cases. Water quality assessed by chemical analyses showed another character of contamination when compared to only manured ponds. These

ponds had much higher contents of nutrients and organic substances than manured and control non-manured ponds. In contrast with manured ponds and in correlation with weather conditions and inflow water quality, high values of heterotrophic plate counts were determined in all seasons (Fig. 2).

Other impacts on faecal ponds contamination

Microbial analyses of fish pond sediments revealed neither faecal bacteria nor pathogens (*Salmonella, E. coli* O157*)* presence in fish pond sediments before and after manuring.

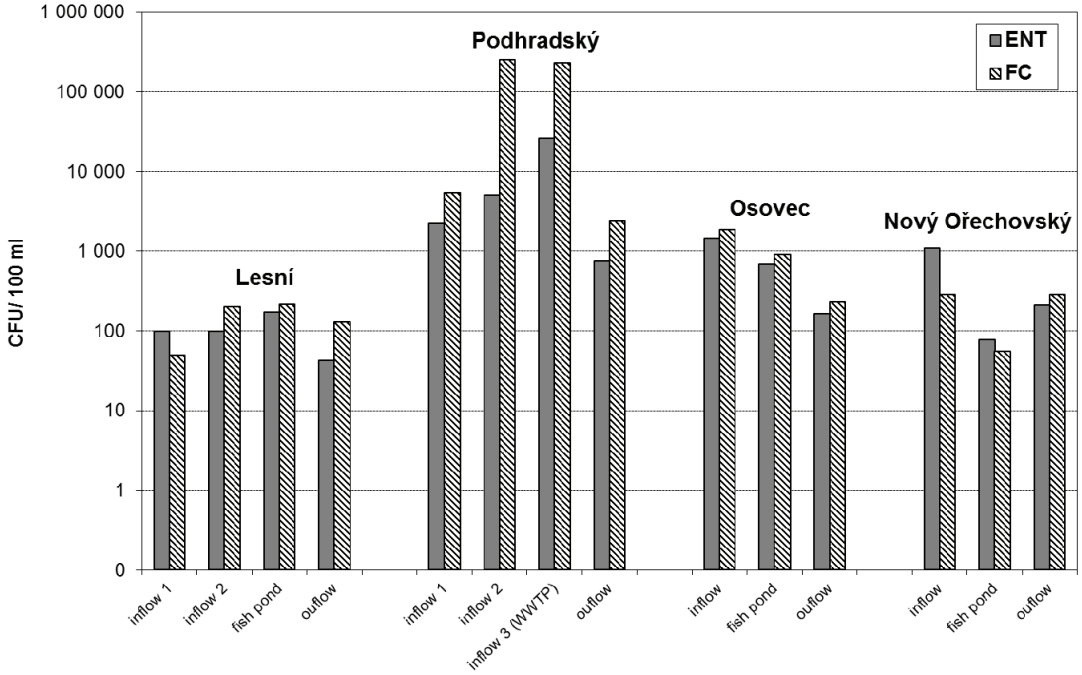
Monitoring of fish ponds-inflows and outflows quality showed usually their very low flow rate or total dried-up in summer months. Nevertheless, some of them served as the significant source of faecal, organic and nutrients pollution (to 10^6 CFU.100 ml^{-1} of faecal indicators). Our results showed that most of the fish ponds work as effective sewage ponds. The water quality improved in all parameters by the passing through the pond (Fig. 3).

Microbiological analyses of the semi-liquid pig manure proved high concentrations of faecal indicator bacteria, 10^4 CFU.100 ml^{-1} of faecal coliform bacteria and 10^5 CFU.100 ml^{-1} of enterococci; *Salmonella* was not detected. The viability of faecal bacteria in the semi-liquid pig manure stored at 8 °C is shown in Fig. 4. Enterococci were detectable in high numbers even after more than two years, while faecal coliform bacteria survived only for 6 months.

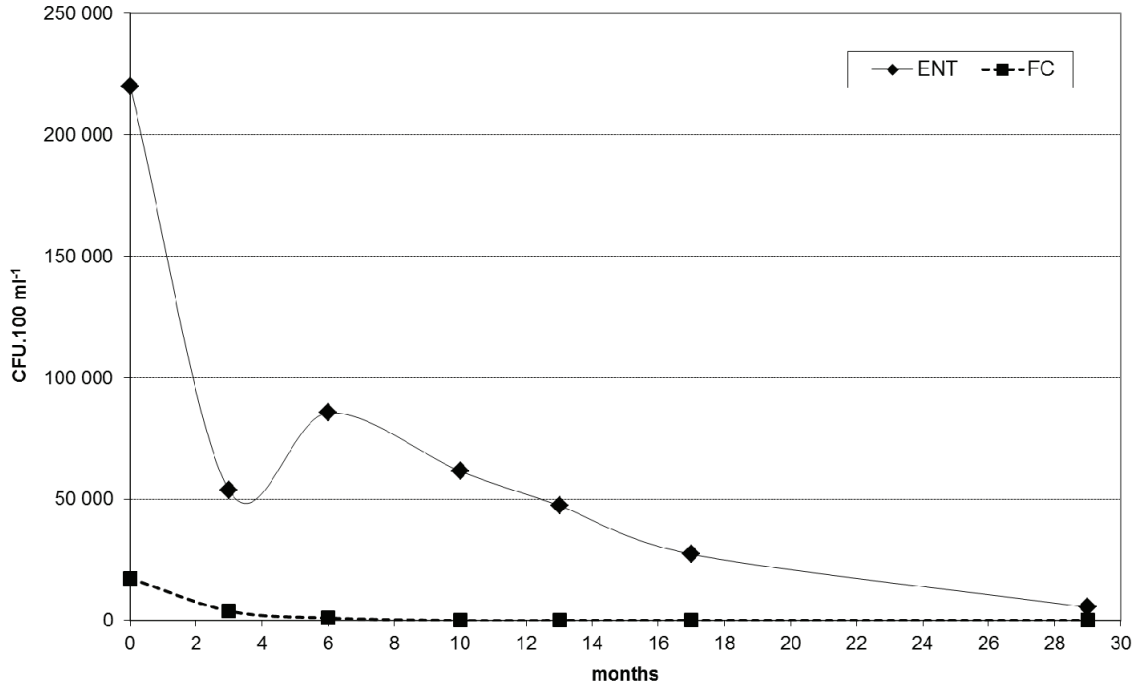
Manuring of fish ponds is one of the basic practices used in inland fishery in order to increase fish pond production. Positive effects on abundance and diversity of phytoplankton and zooplankton and on fish yield have already been described (Govind *et al*., 1978; Dhawan and Toor, 1989; Terziyski *et al*., 2007). The level of fish pond production depends on the quality and quantity of applied manure, the pig manure is thought to be the most effective (Dhawan and Kaur 1978; Dhawan and Toor, 1989). Nevertheless, information about the effect of manuring on water quality and faecal contamination are scarce.

Our results showed that differences found in microbial and chemical water quality of fish ponds depend on their management. The lowest contents of bacteria were found in the fish ponds before the manure application. The state after manure application was usually very similar as for the non-manured fish ponds. The results showed that the heterotrophic bacteria were in short time incorporated into the food chain and that the conditions were not suitable for faecal bacteria growing. The worse water quality and the highest counts of faecal and organic bacteria were found in ponds with incompletely-controlled management, i.e., manuring with municipal waste water inflow, waterfowl breeding etc. In these cases, the potential for optimal nutrient levels and functionality of the natural food mechanisms in fish ponds were decreased. Compared to this, a better situation was in manured ponds with municipal waste water sources, in which the manuring could help to the regulation and balance of the inflow loading pollution.

Nevertheless, the characteristic values of microbial faecal indicators (Fig. 1) were not significantly different in the non-manured and manured fish ponds. However, high faecal indicator counts were



3: *Faecal contamination (faecal coliform bacteria – FC, enterococci – ENT) of fish ponds-inflows and outflows*



4: *Survival of faecal coliform bacteria (FC) and enterococci (ENT) in semi-liquid pig manure*

sporadically detected in summer months. It can represent a potential health risk by recreational use of fish ponds. The strict water quality limits for bathing water (i.e. 300 CFU.100 ml^{-1} as the seasonal mean value for *E. coli* and 100 CFU.100 ml^{-1} for enterococci) given in Czech Government Decree in the evaluated period were exceeded in more than 30% of individual values, but not in seasonal mean value.

The occasional summer increase of microbial indicator counts was not plausibly explained in agreement with our presumptions. Neither the influence of inflows nor release of bacteria from sediments, which were examined as possible reservoirs of surviving faecal bacteria, were proved to be sources of short-term faecal pollution. This result is not in agreement with Šimek and Straškrábová (1989) who found that sediments may act as the reservoir of higher counts of indicator faecal bacteria which could be from the sediment released, for example by the activity of benthivorous fish in summer months.

Integrated livestock-fish farming may be associated with the risk of contamination of pond water and sediments with pathogenic bacteria from the manure. Presence of *Escherichia coli* O157, *Salmonella*, *Campylobacter* and *Listeria monocytogenes* in semi-liquid pig manure and pond water was observed most often (Pell, 1997; Abulreesh *et al.*, 2004; Nicholson *et al.*, 2004). In our study, the presence of potential pathogens in manure, manured fish ponds water and sediments was detected by microbial indicator assessment and directly by *Salmonella* and *E. coli* O157 detection. Our findings of pathogens in manure, sediment and water samples were negative. The survival of faecal indicator bacteria in the semi-liquid pig manure for several months was proved. In case of improper manure handling, possibility of long persistence of enteric pathogens and high probability of their transfer into pond water, where they could serve as the source of infection, can be expected in such environment (Jones, 1976). On the other hand, it was proved that microbial pollution coming from inflows and manure was largely eliminated in all fish ponds before reaching the outflow. This effect, probably caused by the strong filtration activity of zooplankton, was described also by Adámek and Jirásek (1989). Moreover, these authors reported that fish ponds have the positive influence on water chemistry, as far as they are involved in self-purification processes. This ability of fish ponds is exploited in waste water treatment for municipal waste water purification.

In our study, the measured values of chemical parameters were related to regulated biological and chemical processes in the fish ponds and were influenced by municipal contaminated inflows, nutrients supply from the manure and duck breeding as well. The values of some chemical parameters in the pond water, especially Biochemical Oxygen Demand and phosphorus, including seasonal maximums, exceeded the quality standards of Czech Government Decree. The fish management on the ponds requires, because of specific character of environmental processes, an exceptional approach in water quality evaluation.

CONCLUSIONS

Our results showed that differences in microbial water quality in fish ponds depend on their management. Differences in running processes are impacted by the nutrients content and their ratios, course of food chain and by environmental conditions. These facts were proved by comparing of more impact types, i.e., manure application, inflow of polluted waters, waterfowl breeding or their combinations.

- The absolute microbial counts determined in our study were unexpectedly low in all of manured as well as of non-manured fish ponds in comparison to other surface waters.
- We found that the heterotrophic bacteria counts were higher in fish ponds after manure application because of input of easy biodegradable organic matter and bacterial inoculum. This effect is the basis of properly functioning cycle of nutrients, microorganisms, plankton and fish by fish farming.
- The counts of faecal indicators were mainly low both in manured and in control non-manured fish ponds. Nevertheless, higher amounts of faecal bacteria were found sporadically in most of the manured ponds in late summer. Increased numbers of faecal indicators could represent a potential health risk in case of recreational use of fish ponds. However, the explicit connection of occurrence of high numbers of faecal indicators, i.e., increase of health risk by manure application was not proved.
- When we use the strict limits of the Czech Government Decree on bathing waters for evaluation of water quality in studied fish ponds, we can see that the individual values were exceeded in more than 30% of non-manured, in more then 50% of manured fish ponds and in more than 80% of fish ponds impacted by other influences than manuring or by more types of contamination (tentative evaluation because of using individual data, not mean value).
- Inflows and sediments were examined as possible reservoirs of faecal bacteria but none of them was proved to be their source. Pathogens (*Salmonella, E. coli* O157) were found neither in fish pond water nor in manure and sediments.
- More than two-year viability of enterococci in the semi-liquid pig manure was proved.
- Results of chemical analyses showed a specific character of pond water, independently on pond utilization, i.e. low conductivity and ammonia content and high content of organic substances and phosphorus, which requires a distinct approach for water quality evaluation for parameters defining organic matter concentration.
- The fish ponds impacted by other influence than manuring or by more types of contamination, i.e., combination of manuring and municipal pollution or waterfowl breeding, were evaluated as the most problematic ones. The reason might be a more complicated regulation of nutrient supply due to the much higher and continuous faecal and organic pollution and nutrients contents.
- The comparison of water quality in inflows and outflows of the fish ponds showed that most of them functioned as efficient sewage ponds. The water quality was improved in all parameters by the passing through the pond.

SUMMARY

Consequently, the microbial water quality of fish ponds varies due to manuring, but the impact on recipients was not proved. However, the related potential health risk in the case of recreational use of fish ponds seems not to be so serious, as one may expect for application of hazardous substances, which manure represents.

Therefore, it was proved that proper and controlled manuring does not cause significant water quality deterioration in fish ponds and surface water recipients.

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