ABSTRACT

Doctoral thesis "Researches regarding the evolution of quantitative and qualitative bacteriological indicators in drinking water in conjunction with physical – chemical parameters" was conducted over four years of study (2003-2006).

This these sums up a number of 268 pages, being illustrated in 45 tables and 113 figures.

Part I – Stage of knowledge - is composed of 81 pages, representing 30,22% of the thesis and consists of 4 chapters in which are presented selected information from 252 bibliographic titles, Romanian and foreign literature concerning the structure and properties of the water, characterization of natural sources of water, pollution and the self-purification potential and the definition and significance of the main indicators for assessing water quality.

Part II - Personal contributions - is carried over 187 pages, representing 69,78% of the total work volume and is structured in 5 chapters, following purposes and objectives. Each chapter is structured in materials and working methods, results and discussion and partial conclusions, thesis second part ending with general conclusions.

The researches has been conducted on a number of water samples taken from local sources (wells and springs) located in urban or pasture range from Iasi county, sources that were used to ensure intake of water for livestock in rural areas and in the steady growth.

Water used to ensure fluid intake in animals, the same as that for human consumption must present certain organoleptic, physical, chemical and microbiological characteristics, in order to fulfill all conditions of water quality, which not affect the health of animals that hazardly consume it.

The need to know the quality of water for animal consumption, it is necessary for themselves, knowing that it affects animals health, but also the productive efficiency and product quality that is recovered from them.

Improper operation of water sources, involves changes in physical, chemical and bacteriological parametres, while contamination persistence is reflected in a high percentage of illnesses in animals.

Water from underground sources is still the most important source used for human consumption and ensure water intake in animals. It often comes from the phreatic layer unprotected of pollution incidents such as infiltrations and leakage from septic tanks and waste platform, chemicalization from agriculture, etc. Pollution of underground water sources is an almost irreversible phenomenon, self-purification of these sources being accomplished in a long time.

Although it is known that water sources polluted by organic substances, ammonium ion, with nitrites and nitrates in general are inconsistent in terms of bacteriological, we have strengthened these claims by digital expressions.

Our researches are a preparatory stage for achieving quality monitoring patterns of water sources used for watering livestock, applying fast, efficient and reduce cost method, in order to obtain quick results, in short, in order to efficiently implement the necessary measures.

The main objectives set for the thesis compilation followed:

- Hygienic assessment of local sources of water used for watering animals;
- Seasonal evolution of bacterial load from local water used for watering animals;
- Seasonal fluctuations in the level of chemical pollution for pollution indicators parameters;
- Fluctuations of bacteriological indicators of pollution correlated with physical and chemical indicators of pollution levels by calculating the correlation coefficients and statistical significance of their determination;
- Determination of genera and species of pollution indicator bacteria, often isolated from local water sources used for watering animals.

In order to prepare the paper, there were used and measured results of physical, chemical and bacteriological tests performed on water taken from local sources (wells and springs) used for watering the period 2003-2006. Analytical methods used were selected so as to obtain easily interpreted results in a short time, easy to apply. Thus, color and appearance changes were made visually, the changes in odor were detected at room temperature and by heating the samples at $60^{o}C$, determine the water temperature was performed using calibrated thermometers, pH determination was performed colorimetricly and using a pH meter, dissolved oxygen was determined by the titrimetric method and modified Winkler method, determination of oxidable organic substances made by oxidation in the acid or basic environment with acid or alkaline potassium permanganate, to determine the total chloride was used titrimetric method (Mohr) and indicative colorimetric

method determination of nitrates was achieved by Griess method and nitrate determination was made using DMP .

Bacteriological analysis have concerned the determination of the total number of mesophilic bacteria (NTG at 37^{o}C) and the number of saprophytic bacteria using serial dilutions technique described in STAS 3001/1991 and ISO 6222/1999, determining the number of coliforms (CT), coliforms faeces (FF) and faecal streptococci (SF) was achieved by multiple tube technique - Most Probable Number determination, according to the methods described and interpretation of results was done according to ISO 8199.

Determining the number of *Escherichia coli*, also involved the use of multiple-tube technique, confirming the presence of bacteria of this specieson the indol production, or on the enzyme β-D-glucuronidase which is combined with 4-methylumbeliferyl β-D-glucuronide (MUG), forming a fluorescent compound in UV light .

To highlight seasonal fluctuations in the level of chemical pollution of local water sources used for watering the animals were analyzed each 226 number of water samples collected both in winter and in the warm.

Following laboratory analysis it was observed that of all water samples examined, 40,26% had various changes of organoleptic characteristics and samples of water with such changes was higher in warm season (42,50%) than winter (37,73%).

Water temperature from local sourses ranged from the narrow limits to the warm winter, with an multiannual average value of 9,52^{o}C in winter and 16,55^{o}C in the warm one. Mean concentrations of hydrogen ions (pH) no exceedances of the minimum and maximum permissible limits for drinking water (6,5 <pH<9,5 units of pH).

In 30,97% of water samples analyzed for nitrogen (nitrite), concentrations were detected over AML (0,50 mg / l) and the share of these types of water decreased from 31,13% in winter to 30,83% in hot season, which demonstrates the increased speed of mineralization of organic reactions in water, a process in which nitrites are intermediate compounds and unstable.

In 52,21% of water samples analyzed for nitrates, were detected concentrations above 50,00 mg / l (drinking water AML). Share samples of water found in such concentrations and multiannual average values increased from winter to the warm, nitrates being stable compounds in water, with cumulative effect, resulting either from oxidation of nitrites, or from infiltrating in phreatic water of water that had washed soil fertilized with nitrogen.

Due to increased household and livestock activities in rural areas in summer months, the share of water polluted with concentrations of 0,50 mg / L ammonium ions, increased in warm

season to 31,66% from 14,15% in cold season, in the same way, evolving the multiannual mean values calculated for this parameter, too.

The same explanation has an increasing evidence of polluted water with concentrations over AML (5,0 mg O2 / l) for oxidable organic substances, registering in the hot season accounting for 25,83% of analyzed water samples, compared with 17,92% in winter.

Although local water sources are less well oxygenated, with a limited contact with the atmosphere, however, multiannual average value recorded in the hot season dropped to 5,20 mg / l, from 5,49 mg / l - average value calculated for analysrd samples water in winter.

Share of water samples that were detected over the total chloride concentrations of AML, was 17,92% in winter and increased to 19,16% in warm season, flow changes from the hot season being responsible for this increase.

Of the 226 water samples analyzed bacteriologically, 56,63% were non-compliant from the bacteriological point of view to use for drinking.

Due to increase rural household activities and multiple opportunities for contamination due to underground water sources, the weights of samples of water contaminated with mesophilic bacteria, total coliform, fecal coliform, *Escherichia coli* and faecal streptococci increased from winter to the warm season, the same path being followed by the multiannual average values.

Of the water samples confirmed to be contaminated with faecal coliform in winter, 84,78% were confirmed to be contaminated with *Escherichia coli* and the hot season, from the total samples confirmed to be contaminated with faecal coliform, 93,33% were confirmed to be contaminated with *Escherichia coli*.

Knowing that faecal streptococci predominate in animal feces and *Escherichia coli* in the human feces, it was calculated the ratio CF / SF recommended by some authors to determine the origin of pollution. The values of this report should be supraunitary where water contamination with human sewage and subunitary where water is contaminated with sewage of animal nature. Values reported FF / SF calculated from water samples from local watering during 2003-2006 showed supraunitary amounts, which would indicate sewage pollution with human nature. But calculating the ratio EC / SF, which should better outline the origin of pollution, mainly subunit amounts were obtained, which shows that water sources analyzed were mostly likely contaminated with animal manure, manure storage directly on the ground in inappropriate places, being the main cause of this effect.

To highlight the evolution of the pollution indicators bacterial number according to the values and physical - chemical indicators, were taken in analysis each of 226 water samples from

local watering, for which were calculated multiannual average values for bacteriological indicators on every concentrations (values) of physical or chemical selected indicators on the criteria of values variation around AML and by the value disperssion on the study period.

A nonzero correlation coefficient, shows that a parameter values vary in a sense to those of another parameter. If the correlation coefficient values approaching a value of 1 or (-1) and the amount is statistically significant at the minimum threshold of 0,05, then we can say that the value of the parameter (eg. Chemical) may be predictive to bigger values large parameters (bacteriological) for change in reasonable or low levels of bacteriological parameter (for inverse variation).

Of water samples identified with organoleptic changes were confirmed as contaminated bacteriological: 67,64% of the samples with different degrees of turbidity, 27,27% of the samples with changes in taste or smell and 94,28% of the samples with sediment turbidity and discoloration, which confirms that the suspensions and sediments are often composed of substrates that confer favorable conditions bacterial survival and development.

After the temperature ranges considered for the study, were observed directly proportional evolution to the number of bacteria pollution indicator, but correlation coefficient values doesn't show that the using of water temperature as predictor of the degree of bacterial contamination of these types of water sources. This was due to the fact that the temperature of the aquatic environment has not varied widely over a year, non intervening mostly in bacterial populations in order to determine their propagation and damage water quality.

Multiannual average values of the mesophilic bacteria and saprophytic number, evolved directly proportional to pH values considered for the study, while the average values of the number of coliform bacteria (MPN CT), the faecal coliform (MPN CF), *Escherichia coli* (MPN EC) and faecal streptococci (MPN SF) showed slight decreases in the range of pH close to neutral. Calculated correlation coefficients had positive values, but does not indicate the use of pH as predictors of the degree of bacterial contamination of water.

Directly proportional relationship, with correlation coefficients values that reject the null hypothesis ($p < 0,05$) were obtained by studying the evolution of the number of mesophilic, saprophytic bacteria and average values of pollution indicator bacteria on concentration ranges considered for nitric nitrogen, ammonia nitrogen and oxidability, which show that these parameters as predictors for the degree of bacterial contamination of water from local watering. Those found above, underlines once again how the pollution of local water sources, outlined in this mode close relationship between bacterial contamination and pollution with organic substances, from bacterial

contamination and pollution with ammonium ion, whose origin is the purine and waste water infiltration through soil pores, of bacterial contamination and pollution with ammonium ions, whose origin is the infiltrations of purine and domestic sewage through soil pores, the bacterial contamination and nitrogen, which while indicating an older pollution sources of water are set compounds in our case means a continuous pollution by decomposing organic matter which is still, of bacterial contamination and nitrogen pollution from oxidation processes and nitrogen substances.

Inverse relationship with values of coefficients of correlation without statistical significance ($p < 0,05$) were shown between the number of multiannual average values and mesophilic and saprophytic bacteria and dissolved oxygen concentrations, which indicates that this parameter does not may indicate the degree of contamination of local water sources such bacteria, the bacteria can adapt to low levels of oxygen. Inverse relationship with values of correlation coefficients with statistical significance, shows that concentrations of oxygen in the water from local sources, even if they are usually smaller, are predictable to the degree of bacterial contamination, but will take into account the depth groundwater, knowing that the deep waters are generally very low oxygen.

On the ranges of concentrations for total chlorine taken in the study, were found directly proportional to the number of developments mesophilic and saprophytic bacteria and pollution indicator bacteria, but the correlation coefficient values obtained, does not show that the total chloride values as predictable for the degree of bacterial contamination. Decreased MPN CT, CF. EC and SF in the range of concentrations above 250 mg / l, doesn't evidence that contaminated water would be bacteriological, but rather that bacteria are sensitive indicators of pollution in waters with high salinity, which does not exclude the presence of pathogens that may be resistant to such salinity waters.

To identify the genera and species of pollution indicator bacteria isolated from local water sources used for watering was considered a total of 50 samples confirmed as bacterial contaminated water, which was isolated from a total of 158 bacterial strains, 77,84% of strains belonging to 22,15% of coliforms and faecal streptococci.

Framing the bacterial genera and species was done using software API Mini bacterial strains belonging to the group of coliforms and biochemical tests commonly used in bacteriological techniques for bacteria found in the second group.

From the coliform bacteria, with a share of 21,51% were identified bacteria of the genus *Citrobacter,* followed closely by bacteria of the genus *Enterobacter* (20,88%), *Escherichia* (18,98%), lowest weight was recorded for bacteria the genus *Klebsiella*.

*From t*he bacteria included in the genus *Escherichia*, *Escherichia coli* has held a share of 90,00%, from the bacteria found in the genus *Citrobacter*, *Citrobacter freundii* was identified at a frequency of 41,17% and from the genus *Enterobacter, Enterobacter cloacae* was identified in 45,45% of the strains. From the bacteria belonging to the genus *Enterococcus,* with a share of 25,70% was identified *Enterococcus cecorum*, followed closely by bacteria belonging to the species *Enterococcus faecalis* (22.85%).

Identification of enterococci that are regularly animal intestinal microflora (*E. cecorum*) and human intestinal microflora (*E. faecalis*), casts doubt on the hygiene of local water sources operated in rural areas and also shows the risk of morbid episodes both outbreaks in humans, as and animals.