An analysis of water samples surrounding swine farms in Timiş County – A practical guide

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Abstract

The most important role in biological soil pollution is allocated to the untreated waste water used to ground's fertirigation from livestock farms, and in particular of swine units. Applying of arbitrary measures, and national and European legislation's non-compliance are main factors that often makes from this issue a public health problem by the great impact it can generate and create in large agglomerations and animals. The diluted manures are able to affect the quality of the environment mainly by: nitrous oxide, ammonia, methane, hydrogen sulphide, volatile organic compounds, etc. and they, being administered in soils, may cause epizootic and epidemiological aspects and also those relating to environmental protection. In this respect it rise the need for all livestock farms to apply appropriate measures for certain manure treatment, different to species of animals and depending on the collection and discharge systems used. This paper is an original research work and it intends to be also a practical guide to follow for those interested in field research of environmental pollution. There are presented current investigation methodologies of water's quality from swine farms vicinity in Timis County. In four chapters are presented: primary water analysis methodology, the determination of chlorides, nitrates and phosphates for each substance being presented methodology, kits and reagents necessary specific results and their interpretation and conclusions for each study. The last chapter was allocated to the description of the potentially polluting compounds determination by GC-MS technique.

Rezumat

Rolul cel mai important în poluarea biologică a solului este al apelor reziduale neepurate provenite de la fermele de animale, în special de suine utilizate pentru fertirigarea solului. Aplicarea unor măsuri arbitrare, nerespectarea legislaţiei naţionale şi europene face ca aceasta să devină adesea o problemă de sănătate publică prin impactul deosebit pe care îl poate genera şi să creeze probleme mari în zona marilor aglomerări umane şi animale. Dejecţiile au capacitatea de a afecta calitatea mediului în principal prin: protoxid de azot, amoniac, metan, hidrogen sulfurat, componente organice volatile etc. acestea diluate şi administrate în soluri adesea poate genera aspecte de ordin epizootologic şi epidemiologic, cât şi cele referitoare la protecţia mediului, fiind necesar ca în toate fermele zootehnice să se aplice anumite măsuri de tratare adecvată a dejecţiilor, diferenţiat de specia de animale de la care provin şi dependent de sistemul de colectare şi de evacuare utilizat. Lucrarea de faţă este o lucrare de cercetare originală care se doreşte a fi şi un ghid practic de urmat pentru cei interesaţi în cercetarea de teren a poluării mediului. Sunt prezentate metodologiile de investigare curente ale calităţii apelor din limitrofia unor ferme de suine din judeţul Timiş. În patru subcapitole, sunt prezentate metodologia analizei primare a apelor, determinarea clorurilor, nitraţilor şi fosfaţilor pentru fiecare substanţă fiind prezentate metodologia de lucru, kiturile şi reactivii specifici necesari, rezultatele obţinute şi interpretarea lor precum şi concluziile pentru fiecare studiu în parte. Ultimul subcapitol a fost alocat descrierii determinărilor compuşilor potenţiali poluanţi prin tehnica GC-MS.

Animal waste pollution of soil and water – Manure impact on the environment

Because soil represents an important part of the biosphere, an important role it is the capacity to support:

- productivity,
- water quality and
- animal health.

Impact of animal waste management from swine breeding farms on bacterial contamination of underground drainage is difficult to assess in the field.

Bacteria in manure can pollute water, soil and vegetation, constituting a threat to the environment and human health.

In the rivers, pathogenic bacteria from manure can be transported by wind, insects and rodents (39).

Animal manure do not exactly correspond to the needs of micronutrients that provide plant growth (18, 51) and migration of phosphorus from soil to surface waters leading to eutrophication and loss of environmental quality (59).

The manure is discharged to collect anaerobic lagoon daily and used for many years for fertigation (31).

Application of manure on the ground for a long time leads to increased levels of potassium, magnesium and phosphorus, causing disturbance on the nutrient profile of soil.

Manure have the potential to affect air quality by downloading N_2O, NH_3, CH_4, H_2S and volatile organic compounds (2, 26, 56, 58, 68).

Soils with high infiltrative capacity will absorb water and related substances such as nutrients and pesticides, absorption realizing faster than soils with low infitrative capacity.

Swine manure are used as fertilizer because they are sources of organic matter.

They are subject to aerobic biological treatment and subsequent to the removal of nitrogen by nitrification (Figure 1) and denitrification as an alternative for nitrogen surplus (3, 13, 23).

The manure is able to carry the phenolic compounds derived from the decomposition of proteins in the intestine of swine, ammonia, nitrite, and surfactants.

Photo 1. Nitrification biofilters in swine farm (73)

Manure application on soil improved water retention capacity of the soil and resistance to compaction (35).

The chemical composition of the soil undergoes changes due to the application of liquid manure and influence factors such as soil texture, time and method of application of manure, rainfall, crop growth, and time of sampling (3).

The addition of salt or additives in swine diets change the composition of manure and they can accumulate in the soil. High levels of salt in the feed can increase the Na content of manure and soil sodium loading (63).

The manure from pigs fed with high-dose of Cu leads to an increase in the levels of copper, zinc, calcium and magnesium in the soil (32, 33).

Bernal and Kirchmann (1992) showed that pig manure can cause salinization in arid and semi-arid areas (4).

Bacteria present in the manure has an increased mobility in liquids tending to contaminate faeces weight evenly unlike solid manure.

Even if animals do not show symptoms, pathogens may be present in animal waste. Although manure samples from some animals are not contaminated, stored manure may contain pathogens from a small number of animals (7, 24).

Bacteria survive a longer period of time in soils with high water holding capacity (21).

Factors that influence the movement of bacteria in soil are:

1) Flow characteristics dependent on the size and structure of the porous soil which

control porosity. We incorporate partition spill water on the soil surface and infiltration as an important factor in the movement of bacteria.
2) The effects of filtration depending on the soil micropores, and the micropores in the upper obstruction of the filters of the solid components of the slurry produced in relation to the size of the microbial cells.
3) Infiltration of the organic material layer formed on the surface, which is a sum of electrochemical filtration and retention in the organic surface.
4) Retention in soil bacterial cells and organic particles by absorption and adhesion strength of ionic substances in soil is very important. Retention is the result of complex interactions between bacterial cells, soil and soil manure solution (66).
5) Microorganisms affect soil quality, soil health indicators are also. Quality is an indicator of sustainable agriculture (27).

Microbiological studies show organic origin or microbiological pollution (12).

The type and number of microorganisms in the animal waste depends on the animal species, age, type of litter storage method (solid or liquid) and storage time (34, 43).

Water pollution and soil may indicate zoonotic agents (tuberculosis, brucellosis, anthrax, tetanus) (6, 49).

Petkov and Baykov (1978) showed that soil at about a meter of manure lagoons collection, regardless of season, are contaminated (number of cultivable microorganisms located between log5 and log6 CFU/g soil) and soil at a distance 30m lagoon are easily contaminated (number of cultivable microorganisms and located between log4 log5 CFU/g soil) (49).

Number of coliform bacteria in stored manure is higher in summer (temperature of 21.7°C) and was also demonstrated and E. coli survives better at 5°C than at 25°C (9, 50).

Gessel (2004) inoculated *Salmonella anatum* in swine manure, completing Johnston (1996) studies who showed that the pathogen survives in the soil for at least 27 days, in correlation with different soil depths (22, 29).

Swine manure is a source of 5% and 3% phosphorus and nitrogen and often this amount exceeds the needs of the plant, so can become a potential pollutant to deep waters and surface waters.

Phosphorus migrates with eroded soil in surface waters, altering its quality (11).

Earthworms in the soil tend to have an impact by reducing *Salmonella enteritidis* in the soil and on the normal bacterial flora in the soil (40),

Fecal bacteria survive a long time after manure application on soil and groundwater after their entry period and could be extended to several months (24).

Studies by Sjogren and Gibson (1981) shows that the lakes, although average diluted ensure viable conditions for bacteria from feces (60).

Water quality is an essential element in the management of agricultural practices.

Scientific studies show that inorganic fertilizers have reduced microbial activity compared with organic fertilizer (15, 36, 46, 47, 52).

The effluents from pigs results in a decrease in pH proportional to increasing the frequency of application to the soil, being influenced by the depth of soil application (15).

Potential pollutant substrate of residues in swine farms

Residues from livestock production cause changes by increases in: content of carbon, nitrogen and phosphorus in microbial biomass, enzyme activity microbial population levels and structures observable (1, 48).

Increased activity or microbial biomass is correlated with an increase in mineral

nitrogen, organic carbon, cellulose degradation and soil organic matter (17, 37, 45, 53, 55).

Pig manure improves soil structure due to the increased amount of nutrients contained: nitrogen and potassium, as well as the disadvantage of having a high content of heavy metals and sodium as potential contaminants.

Agricultural efficiency and reducing the risk of environmental pollution is the effective management of manure.

The consequences of using swine manure as part of soil fertilization lies in the quality of organic matter, nutrients, micronutrients and other additional factors such as food additives, health and pharmacological products.

Manure from animals is an important factor in the spread of disease so that animal waste assessment algorithm aims to protect water and soil hygienically and epizootic (30, 45, 66, 67, 70).

Microorganisms in the soil mass turns manure into humus, changing living conditions of pathogenic and potentially pathogenic bacteria (50).

Bacteria infiltrates the soil is dependent of the soil physical configuration, soil chemistry and properties of microbial cells, dependent on the fluctuation of macropores and particle interaction.

Changes in soil affects the interaction between bacterial cells and soil in many ways:

- increasing filtration,
- changing the physicochemical interaction kinetics between charged surfaces and
- changing competition on fixing sites between soluble and powders components.

Research carried out over the waters of the wells showed that the percentage of wells contaminated with faecal bacteria is grown in farms where manure is spread on land than farms that use mineral fertilizers, showing that manure is an outstanding source for nitrate and bacteria contamination of wells.

E. coli and Enterococcus spp from soil manure can survive even 40-68 days after application.

Survival depends on the source of microbial species, and the method of application of the slurry.

In the solid manure deposits are charged different temperatures due to organic matter digestion (aerobic to the periphery tending to anaerobic to the center).

Microorganisms have survival rates in these areas, in the near periphery are more likely to survive and form the source of contamination (66).

Pollution by nitrates and nitrites on environmental homeostasis

In some of manure nitrogen is found in the existing complex protein molecules of the digested feed.

Ammonia is the most accessible to the plants after its conversion into nitrate by bacteria in the soil.

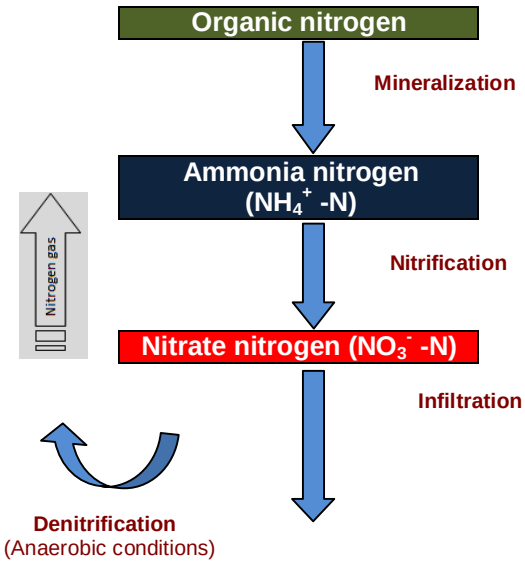
For nitrogen management is necessary to know the amount of ammonia nitrogen from total nitrogen.

To avoid ammonia volatilization losses of nitrogen incorporation is required and not to apply manure on land surface without incorporation.

Factors which influence the degree of volatilization are:

- air and soil temperature,
- atmospheric humidity and
- air currents when applying,
- application procedure and period
- correlated during optimum takeover of nitrogen by plants (65).

Temperature is an important factor, because the warmer soil is the faster will help transform ammonia nitrogen to nitrate (19).



Scheme 1. Nitrogen transformation in soil (19).

Manure application on soil highlights nitrogen increasing the amount disturbing soil nitrogen balance.

The effect of using swine manure as fertilizer are important for the oxidation of ammonia nitrogen in the soil investigation (8, 16).

Although there are three types of soil nitrogen plants may use only two:

- ammonia and
- nitrate.

Plants can use organic nitrogen by soil microorganisms intervention.

When applying liquid manure to the soil, the two forms of nitrogen pass through the low depth soil followed by transformation of ammonium nitrogen to nitrate.

Elevated nitrate concentrations have destructive effects in deep waters and are interdependent with the rainy period.

It tends to acquire high concentrations in dry areas because of the dilution effect is reduced. Excess nitrate in water can accelerate the growth of algae and plants in lakes and streams, leading to depletion of oxygen supply.

Nitrate concentrations in drinking water can affect young animals or small children. Increased nitrate in drinking water causes methemoglobinemia, or blue baby syndrome, occurring in children under six months (28).

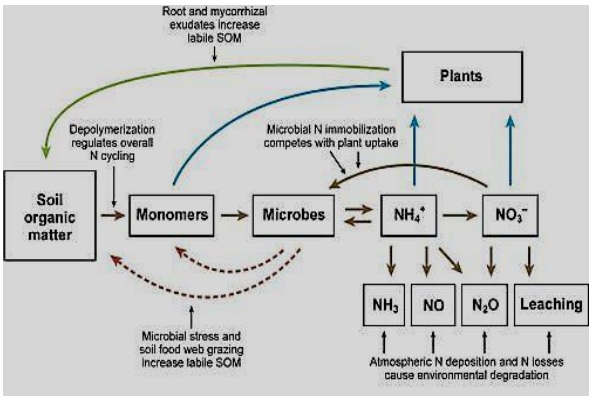


Figure 3. Nitrogen reactions in soil (27, 57).

Spalding and Exner (1993) shows that consumption of water contaminated with nitrates can be correlated with hypertension, congenital, infantile form tumors and mortality (61).

Multi-organ disorders of nitrates begin irritating and then congestive of the digestive tract mucous with manifestations gastrointestinal diarrhea.

Irritant effect to the kidneys is manifested by polyuria and hematuria.

By reducing the oxygen binding capacity, a reduced tissue respiration and oxidative phosphorylation occurs and hypoxia and anoxia and then being outlined it's methemoglobinizant effect.

Physical organic and functional disorders of the action of nitrate are represented by severe respiratory failure, tachycardia, apparent mucous gray - brown - yellow, red- chocolate blood color.

The nervous system is severely affected by paralysis of vasomotor centers, producing vasodilation of small caliber vessels, with hypotension and collapse.

Acute and superacute form evolves with asphyxia signs.

Chronic form occurs due to the methemoglobinisant limited effect, tenuous symptomatic: hypothyroidism, reduced

deposits of vitamin A, expressed by reduced growth and milk production.

Reproductive impairment is of great interest and can generate economic damage highlighting events like: repeated breeding, difficult to treat endometritis, abortions.

Multi-organ lesions initially begin by congestion followed by degeneration in the liver, kidney, brain, lung, heart, testes (65).

Forage plants can have a nitrate content dependent of factors dependent on plant (species, plant part and stage of maturity), how to harvest and conservation measures and the climatic conditions.

Although nitrates accumulate especially in underground parts of the plant, there is a tendency to accumulate nitrates: beet, barley green, green oats, corn, rye, sorghum, millet, alfalfa, soybeans, wheat.

Also, increased amounts of nitrogen can meet at the bottom of the strain.

Depending on the stage of development, we can say that the amount of nitrogen is increased in young plants often reduce those at maturity and increase only if the climatic conditions are favorable for accumulation.

Humidity is a crucial factor because after the rain increases the amount of nitrogen in plants.

Due to the development of reducing bacteria the nitrate grows mostly by chopping and keeping piles of green fodder.

An effective labor reduction of nitrate levels of up to 40-60% is silage.

Consequences of nitrates and nitrites are seen mostly in young animals due to their oxidizing action on fetal hemoglobin.

Animal feeding mode affects toxicity, especially mixed feed low-risk compared to uncontrolled feeding of animals.

Mixing feed standardizes the amount of nitrate ingested per 24 hours.

The use of concentrates in the ration gives energy intake and reduces the toxic effect of nitrates.

An accumulation of nitrates may occur due to deficiencies in micronutrients involved in the conversion of nitrate to ammonia (65).

Deng (2006) showed that after using anhydrous ammonia increased while the amount of nitrate reduced chemical and microbiological activities (15).

Manure increase the amount of nitrate (20, 42), enzymatic activity (47) and microbial (46, 51).

Microbial metabolism is reduced due to food deprivation, low temperatures and low levels of water availability.

Even some minor environmental changes can lead to reactivation and acceleration of bacterial cell multiplication (66).

Soil microorganisms such as protozoa, nematodes and *Bdellovivrio* bacterium kill bacteria in the soil and hence those introduced along with manure (25).

An excess of nitrogen-containing products leads to the release of potential pollutants in the air, which has consequences for the discharge of nitrates in water, becoming a major risk factor for public and animal health.

Cromwell points out that nitrogen is present in pig manure due to not using digestible protein or loss of body protein.

There are several methods to reduce the excretion of nitrogen by reducing the levels of proteins ingested with the feed as the excess of amino acids in the protein will then be converted to urea and excreted (11).

Sources of digestible protein in feed lead to massive excretion of nitrogen.

By maintaining the ambient temperature of the shelters in the comfort of pigs and disease control can improve feed conversion and also reduce nitrogen excretion (11).

Due to the intensive use of manure in the soil fertilization is the possibility of increased amounts of residual nitrogen in various forms, discharge of nitrates in water or ammonia emissions (15, 71, 72).

The enzymatic activity in the soil and biotransformation processes are often related to the pH of the environment.

Table 1.
Available nitrogen in the soil correlated with the number of days between application and incorporation into the soil (65)

Available nitrogen %		Timing of application (month)	Days to incorporation
NH_4	Organic		
50	33	11-02	<5
25	33	11-02	>5
50	33	03-04	<3
25	33	03-04	>3
75	33	05-06	<1
25	33	05-06	>1
75	15	07-08	<1
25	15	07-08	>1
25	33	09-10	<1
15	33	09-10	>1

The biochemical process of organic nitrogen mineralization include various enzymes L-asparaginase, L-glutaminase, amidase and urease (15, 64).

Swine effluent treatments did not significantly disrupt the L-asparaginase activity and L-glutaminase but reduce urease activity and amidase (15).

Biochemical and microbiological activities that relate to the nitrogen arid agro-ecosystems cycle depend on the treatment carried out on land and also the carbon efficiency.

The content of the carbon in slurry can be correlated with the dry matter content. Increased levels of ammonia combined with low carbon liquid manure in the environment is reflected by the abundance of nitrogen (66).

Agro-ecosystems sustain agricultural production while preserving environmental quality (15).

Țibru (2009) highlights the elements needed to prevent pollution human-animal-environment triad:

- standardized use of nitrogen fertilizers, the determination of the content of the feed before introducing consumption and from drinking water;
- management of components with high nitrogen content in the form of concentrated;
- silage should be made only after completion of fermentation, feed with high nitrate-nitrite ration will be introduced gradually, balancing rations in terms of protein-vitamin-mineral;
- receiving treatment before and after absorption: activated charcoal, saline purgatives, methylene blue, vitamin C, adrenaline, caffeine, glucose dependent on the symptoms appeared (72).

The role of heavy metal in environmental pollution

Toxic metals may be present in wastewater from pig farms, that can have a negative impact on the environment.

Steinmetz (2009) noted that the metal concentration in the pig manure time-varying output dependent on changes in their food, and taking into account the number of animals (62).

These metals originate mainly from feed since many inorganic salts added to the ration, not only as essential nutrients but also as a supplement for improving health but also for feed conversion efficiency.

A high amount of metals ingested by pigs is excreted in urine and faeces and therefore the concentration of metals present in manure is dependent primarily of constituents of the ration.

A 90% Cu percent ration administered is found excreted in the faeces (33).

Between the amount of copper and zinc in the ration and the on in manure there is a direct relationship of proportionality in that there a higher dose in the ration means an increased presence in manure (41, 69).

Arsenic, cadmium, chromium and iron are administered as metabolic markers for weight gain of the animals, although they are toxic above certain concentrations that can have negative impact on microbial populations in aquatic environments (5).

Use of slurry increases the availability of carbon substrate and mineral nutrients, such as ions of ammonium, phosphate, potassium, sodium, magnesium, calcium, metals such as zinc, copper, changing the

rate of survival of the bacteria and stimulating the biological activity of the soil (66).

Pollutant character of manure

Manure management is necessary to have annual fertilization plan for each farm based on agrochemicals studies, crop structure, number of animals, method of storage, processing technique and the method of dispersal of garbage.

It is not indicated the application of manure on the soil during September 1 to February 1 on hay or cropland crops during fall and between August 1 to February 1 on different grounds.

In determining the storage capacity of a farm will consider a rate of 6% of the live weight of the animal as the amount of manure per day resulting from a pig, regardless of category.

Capacity storage tanks or lagoons must be greater than 2.5 volume of manure removed.

This capability is necessary in order to overcome the six months of storage required, and to maintain the slurry under optimum conditions in view of the fact that due to the fermentation process increases the volume of slurry during storage.

Excess nitrogen in the soil occurs due to: the massive application of nitrogen fertilizer or organic fertilizer, high temperature favoring the action of bacteria in soil nitrification and soil molybdenum deficiency (30, 65).

Existing objectives in the application of manure on the soil surface are: ensuring maximum utilization of nutrients by vegetation and minimize the chance of water pollution.

Factors that limit the amount of manure to be applied on agricultural land are: bearing capacity of the soil, the nutrient content of manure, crop nutrient requirements, limited space, the slope of the potential for washing and leaching potential.

Factors dependent on management that may prevent manure entering watercourses are:

- constructing a gang buffer along the watercourse in the right portion where the potential for leakage of manure in the water is high, can reduce the amount of manure that gets into the water;
- soil surface condition is important for uneven ground surface or covered by reducing the degree of leakage of manure in the water compared to soil smooth or bare;
- characteristics of the slurry, the rate of application, method of application influences the liquid manure applied in amounts greater than the rate of infiltration into the soil or the water retention capacity of the soil may facilitate slurry flow;
- soil nutrient status before applying manure requires an increased amount of nutrients favoring nutrient losses after manure application to a greater extent than when the quantities are small;
- surface or underground drainage covers drainage installations running can reduce the potential for leakage or direct discharge of manure applied to agricultural land.

Risk factors for soil pollution by nitrates are:

- water permeability of soil and water retention capacity;
- the nitrate content in the soils and crops quantities taken;
- practiced method of irrigation and the amount of water used for irrigation.

Land with maximum capacity of nitrate pollution of soils are coarse and medium coarse texture which have high permeability and low water retention capacity. It is higher in cases where the water table is located at shallow depths (about two meters) and regularly cultivated land applying high nitrogen fertilizer doses.

Irrigated land which prevails coating medium and fine textured formations and

groundwater level below two meters is characterized by a reduced risk of nitrate dissipation into the environment.

Table 2.
Agro-soil and organizational measures necessary to avoid water pollution (44)

Organizational measures	Agro-soil measures
arrangement of land against erosion and avoid erosion by irrigation	adaptation of appropriate irrigation methods with soil and topography, with the quantity and quality of available water, with crop requirements and climatic conditions in the area;
exclusion from irrigated agriculture of land at risk of erosion and solifluctions	avoid compacting the surface and shallow water which can cause puddles and runoff formation;
avoid overloading phenomenon with nitrate of soil with erosion risk	avoidance of formation of under irrigated and moderately strong bands compacted favoring formation runoff and hypodermic;
avoid spillage of the irrigated surface and material moving in waters;	exclusion of soil physical degradation processes and avoidance of excess water areas where runoff may occur;
the choice of technique and quantity of irrigation water applied according to the soil characteristics;	preventing the phenomenon of cracking of the soil deep preferential pathways leading to the occurrence of flow of the water, excessive increase in the first stage of the infiltration rate of water and therefore the loss of water from the active layer of the soil, affecting the hydrologic regime. Thus there is the potential leaching of nutrients and pollutants in groundwater, contributing indirectly to pollution and changes deep nutrition regimes;
irrigation application as evenly as possible to avoid the formation of excess water areas where runoff may occur, leveling land and ensure uniform distribution of water over the soil surface avoiding surface runoff;	on soils with high permeability is contraindicated gravitational irrigation. On such soils it is recommended localized drip irrigation or minisprinklers;
when irrigation is such that an easy crop to suffer from water deficit, because in such a situation, the applied water is consumed very intense;	
stimulation of a well developed root system, able to explore a larger volume and greater use of water and nutrients;	

Stabilization and disposal of animal waste

Discharge of wastewater and excreta in rivers and ponds fish may cause poisoning or weaken resistance of fish and rapid evolution of infectious dropsy of carp.

Taking into account both epizootic and epidemiological order and those relating to environmental protection, it is necessary that all livestock farms to apply certain measures for appropriate manure treatment, differentiated from animal species and dependent on the collection and evacuation system used.

Slurry stabilization may be achieved by speeding up the processes of digestion, switched off or inhibition of microbial degradation for the slurry to be introduced into the economically useful biological cycle (14).

Stabilizing clean manure in swine livestock farms

Manure stabilization by aerobic fermentation is a process used in farms showing hydraulic discharge of manure, covered with grates and channels that do not have sufficient space home of a waste.

The degree of aeration of the manure in channels is important because it determines the speed of oxidation-reduction process.

Basal aeration is similar to basal artificial aeration of the aquarium using a network of perforated tubes located at the bottom of the manure channel through which air is introduced under pressure.

The air bubbles maintain slurry in continuous suspension and promote aerobic bacterial growth.

The effectiveness depends on the size of bubbles and the thickness of the slurry, which influences the degree of oxygenation of the environment.

The system requires constant supervision and can not be applied to houses with large capacity.

Surface aeration is to introduce air forcibly into the layers of the slurry or the design into water of the sewage water.

Aerator with suction and turbo-mixer aerator determines a good aeration and circulation in semi-continuous slurry channels.

The aerator is composed of a propeller attached to the end of the tube and mounted directly to an electric motor.

Air is drawn through the tube and conveyed as a stream in the liquid by the action of the propeller, at an angle.

A single aerator can be enough to shelter 180 pregnant sows, 60 sows with piglets, weaners, 360 or 180 fattening pigs.

To use this ventilation system is required channel coupling two by two semi-circular channels of communication at the ends, so as to obtain one or two complete circuits.

Removing stabilized waste can be done by drain or roof drains overflow channel located at the tip. he waste stream is collected in tanks situated outside the housing, where rapid sedimentation occurs, and the separation of the liquid phase from the solid.

The solid phase can be conducted to the drying beds, and a part of 35% can be reintroduced into the aeration channels to their aerobic reseeding. Liquid phase can be sent to a farm outside the lagoon or in a biological pond where continuous natural self-purification processes.

From this treated water is discharged safely into an emissary used for fertilization or for periodic washing of the channel and at need to evaporate the water filling the channels, so as to maintain a constant ratio between the water channels and the dry matter.

From the economic point of view, it is advantageous to stabilize the residues in the channels, with a good aeration of the organic matter transformation is performed into microbial protein slurry, the development of mixed cultures of bacteria, actinomycetes, fungi and protozoa (up to 200,000 protozoa / ml). This ensures the preservation of an important part of the nitrogen in the manure, which would be lost as ammonia, if anaerobic fermentation (14).

Wastewater treatment methods

Primary treatment (mechanical)

Makes the separation of pollutants based on physical separation processes: sedimentation (natural decantation).

Therefor such retention occurs of floating bodies and sediments settle. Natural sedimentation allows settling particles with diameters greater than 0.1 mm, and the equipment used are, in order of the paths followed by water entering the station, grills, site, desanding and primary clarifiers.

The grids and screens to remove coarse material larger than 1 mm at a water speed of 0.3-1 m/s. By gravity decantation in desandings settling in a water velocity of 0.3-0.4 m / s, the particles are sedimented with a diameter over 0.1 mm, over the course of 2-3 minutes.

The primary sedimentation tanks are subject to the remaining part of the suspension decanted and colloidal substances, water is retained for 2-3 hours.

Mechano-chemical treatment removes pollutants as unsettled sediments, remained in matter unsettled.

Such other pollutants are converted into substances, easy to separate noxious or low likely to be removed by other purification processes.

The most common processes are:

- neutralization,
- oxidation-reduction,
- precipitation,
- clotting (flocculation) and
- ion exchange.

Separation of unsettled sediment by chemical processes involves treating water with coagulants (aluminum sulfate, ferric chloride, chemicals macromolecular).

Resulting flocs have a higher specific weight and will quickly settle into the decanter.

Biological treatment removes biodegradable organic pollutants.

Organic substances can be removed from water by microorganisms that use them as food, like a carbon source.

The most common treatment option in which microorganisms are suspended in water in the form of flakes is the activated sludge process.

The waste water is introduced in an aeration basin containing a suspension of biological flocs in which is used the oxygen required for breathing. In addition to bacteria, algae multiply in these basins, after photosynthesis release oxygen and avail nitrogen in the water contributing to water purification (54).

Classical methods of soil decontamination

Decontamination by biodegradation relies on existing microorganisms (bacteria, fungi) to decompose organic pollutants containing carbon is recommended for soils with high organic pollutants: phenol, polycyclic aromatic hydrocarbons.

Biodegradation loose is basically a soil decontamination by composting operation, achieving excavating contaminated soil and its arrangement in the vicinity of the excavation in order to initiate and conduct natural aerobic biodegradation process.

Bacterial leaching is the extraction by bioleaching of the metal elements from polluted soil. This separates the contaminated pollutants fron the environment without destroying them.

Bioaccumulation

Performs decontamination of soils polluted with heavy metals.

Passive bioaccumulation is carried out by fixing polluting elements at the surface of certain micro-organisms or plants.

Active boaccumulation is the uptake of pollutant by the microorganisms and plant cells. Bioaccumulation is considered active process by which the concentration of pollutants inside living cells is greater than the outside (54).

Water pollution study
1. Collection and primary analysis of water samples

Water samples were collected in plastic containers, opaque white, and were stored in a refrigerator at a temperature of 4^0C to time of the measurements.

Test samples used were from streams taken from different locations: Pădureni, Parța, Voiteni, Peciu Nou, Ciacova, Chevereșu Mare.

Sampling was done with recording GPS coordinates, time of sampling, the water temperature at harvest, ambient temperature, barometric pressure, weather condition, relative humidity, conductivity, salinity, pH and dissolved oxygen (Table 1, Figures 1 -5).

Establish sampling locations was made dependent on swine farms neighboring regions to determine a possible pollution (Figure 6).

Figure 1. Device for detecting temperature and YSI model 55 dissolved oxygen meter

Oxigenmeter YSI55 - measured in field conditions dissolved oxygen with selectable percentage or mg/L using a static polarographic sensor with a sensitivity between 0-20 mg /L (0-200% air saturation), accuracy ± 0.3 mg/L (± 2% saturation).

Figure 2. Apparatus for determination of dissolved oxygen Elmetron model CO-401

Oxigenmeter Elmeron CO-401 has a sensitivity range-between 0-60 mg / L (0-600%) and an accuracy of ± 0.1 mg / L (± 1%).

Figure 3. Apparatus Orion model 115

Conductivity meter Orion 115 has a sensitivity of 0-1999 µS and accuracy ± 0.5% (to determine conductivity), a sensitivity between 0-80% and an accuracy of ± 0.5% (for salinity.

Figure 4. The technique for determining the conductivity in field conditions

Conductivity meter Orion 115 presents the following parameters to determine the temperature: -5 to-105^{0}C sensitivity, relative accuracy ± 1^{0}C and resolution 0.1^0 C.

Figure 5. Ph-meter: ph/ EC/ TDS Waterproof Family used in the experiment (Hanna Instruments)

Ph-meter EC/ TDS Waterproof Family with an accuracy of ± 0.5^0 C temperature and pH of ± 0.01.

Table 1.
Physical and electrochemical parameters of water samples

Water samples	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
GPS coordinates of the location	Long. 45°62'75" Lat. 21°1' 30"	Long. 45°40'0" Lat. 21°28'59"	Long. 45°45'6" Lat. 21°1'05"	Long. 45°45'6" Lat. 21°1'66"	Long. 45°46'66" Lat. 21°1'33"	Long. 45°51'65" Lat. 21°1'49"	Long. 45°46'66" Lat. 21°1'33"	Long. 45°45'6" Lat. 21°1' 66"	Long. 45°51'65" Lat. 21°1'49"	Long. 45°45'6" Lat. 21°1'05"	Long. 45°62'75" Lat. 21°1'30"
Sampling hour	10.30	9.20	10.58	11.15	11.35	12.05	12.10	12.35	13.02	13.40	14.15
Water temperature	8,2^{0}C	7,3^{0}C	7,1^{0}C	7,0^{0}C	6,4^{0}C	7,8^{0}C	9,7 ^{0}C	8,1^{0}C	9,4 ^{0}C	8,8 ^{0}C	6,9 ^{0}C
Ambient temperature	3^{0}C	2^{0}C	3^{0}C	3^{0}C	3^{0}C	3^{0}C	7^{0}C	7^{0}C	8^0 C	7^{0}C	7^{0}C
Atmospheric pressure	1048 hPa	1048 hPa	1048 hPa	1048 hPa	1048 hPa	1048 hPa	1012 hPa	1012 hPa	1012 hPa	1012 hPa	1012 hPa
Weather	Light rain	Light rain	Light rain	Light rain	Light rain	Light rain	Cloudy sky	Cloudy sky	Cloudy sky	Cloudy sky	Cloudy sky
Relative humidity	98%	98%	98%	98%	98%	98%	63%	63%	63%	63%	63%
Conductivity							466 µS	538 µS	737 µS	509 µS	109,2 µS
Salinity							0,2 ‰ 221 mg/L	0,3 ‰ 255 mg/L	0,4 ‰ 351 mg/L	0,2 ‰ 242 mg/L	0,1 ‰ 51 mg/L
pH	7,5	7,6	8,7	8,2	8,3	8	7,1	7,5	7,7	8,6	7,9
Dissoved oxygen	4,9 mg/L	6 mg/L	4,4 mg/L	4,7 mg/L	5,9 mg/L	4,7 mg/L	7,34 mg/L	4,62 mg/L	3,45 mg/L	3,75 mg/L	4,66 mg/L

Legend:
P1 = Parța; P2 = Chevereșu Mare; P3 = Peciu Nou; P4 = Pădureni; P5 = Voiteni; P6 = Ciacova; P7 = Voiteni; P8 = Pădureni; P9 = Ciacova; P10 = Peciu Nou; P11 = Parța

Figure 6. Establish sampling locations dependent on swine farms in neighboring regions

2. Determination of chlorides in water

It was used testing kit HI^{3815} produced by Hanna Instruments.

2.1. Testing kit components

Materials included in the kit (figure 7):

- difenilcarbazone indicator, a bottle with dropper (15 ml)
- solution of nitric acid, a bottle with dropper (30 ml)
- mercuric nitrate solution HI3815-0, a bottle (120 ml)
- two calibration pots (10 and 50 ml)
- calibration syringe tip.

Figure 7. Kit for determination of chloride HI^{3815} (Hanna Instruments)

2.2. Testing utility

Specifications: The method of analysis was a mercuric nitrate titration. The amount of the sample was between 5 ml and 50 ml.

Importance: Chlorine ions constitute a majority of inorganic anions in water and wastewater. Although there is no known toxicity of high concentrations of chlorides in humans, adjusting the concentration depends on the taste. It is essential to monitor the concentration of chloride in boiling systems to prevent degradation of metal parts. Too elevated levels of chloride has corrosive effect on stainless steel and can be toxic to plants.

2.3. Chemical reaction

The level of chloride in mg / L (ppm) was determined by titration of mercuric nitrate.

The pH is brought to 3 by addition of nitric acid. Mercury ions react with chloride ions to form mercuric chloride.

In the presence of an excess of mercury, they formed a complex with difenilcarbazone resulting in a purple solution.

Change in color was made from yellow to purple highlighting the final moment of the titration.

2.4. Mode of action

There were placed 5 ml of the sample into a calibration vessel and in the holes in the cover of the calibration vessel were added two drops of indicator difenilcarbazone, homogenizing in a circular motion of the vessel.

The resulting solution had a red-violet color. There was then added nitric acid solution, stirring continuously the bowl, until the yellow color appeared (9, 10).

In the titration syringe it had to be taken an amount of mercuric nitrate solution until the HI3815-0 0 ml graduations on the syringe barrel was reached, then it was added the solution of titration into the vessel of calibration lid orifice, stirring to mix after each drop.

The maneuver was continued until the transfer of color from yellow to purple occured (Fig. 8).

The reading of graduation in milliliters of the titration solution was on the syringe scale and multiplied by 1000 to obtain the chloride in mg / L (ppm).

2.5. Results and discussion

Results obtained are shown in Table 2.

From the data it is observed that after repeating measurements in different weather conditions (humidity 100% and cloudy weather without precipitation), water chloride values differ significantly, so that in four of five locations recorded increases in chloride and in one location values are lower in the second determination (Figure 1).

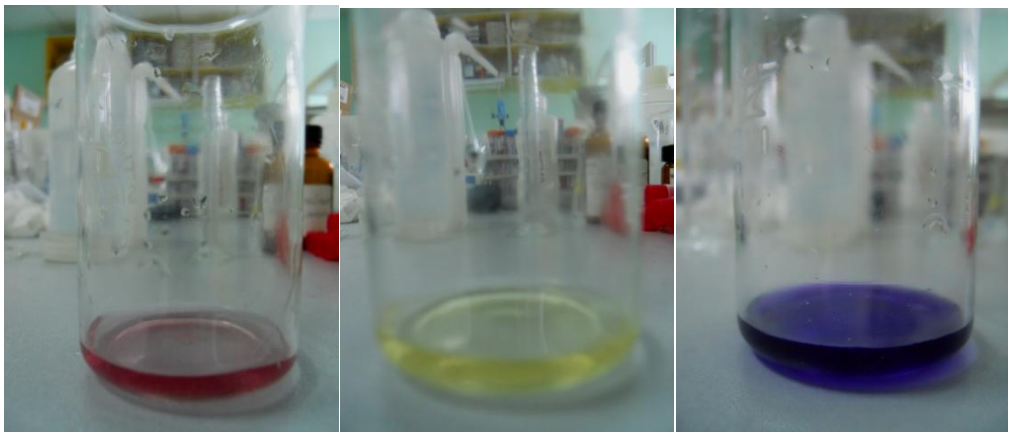


Figure 8. Turn color during testing

Table 2.
The amount of chloride in test water

Water sample	Level determined by testing chlorides (ppm)
P1 (Parța)	38
P2 (Șurgani)	30
P3 (Peciu Nou)	21
P4 (Pădureni)	31
P5 (Voiteni)	65
P6 (Ciacova)	12
P7 (Voiteni)	50
P8 (Pădureni)	100
P9 (Ciacova)	120
P10 (Peciu Nou)	130
P11 (Parța)	150

The maximum of chloride was recorded at the (150 ppm) and minimum at Ciacova (12 ppm). All results concerning chlorides in water samples fall within the maximum allowable under the law in force (250 ppm, representing the limit of chlorides in accordance with Law no. 458 of 8 July 2002 on the quality of drinking water supplemented by Law no. 311/2004) (82).

For determining the dissolved oxygen in the laboratory was used HI3810 kit produced by Hanna Instruments (Figures 9-10).

Figure 9. Kit for the determination of dissolved oxygen HI3810 (Hanna Instruments)

Table 3.
Quality parameters in fish ponds over several months in a row

Elements / Months of testing	Jan.	Feb.	Mar.	Apr.
Total organic compounds (mg/L)	35,1	35,5	47,2	108,7
Turbidity	0,496	1,63	0,509	0,77
Dissolved oxygen (mg/L)	11,1	11,7	11,3	8,7
Temperature (^{0}C)	9,4	10,7	12,7	20
pH	7,15	7,13	7,2	6,62

Graph 1.
The amount of chloride on the basis of the sampling site

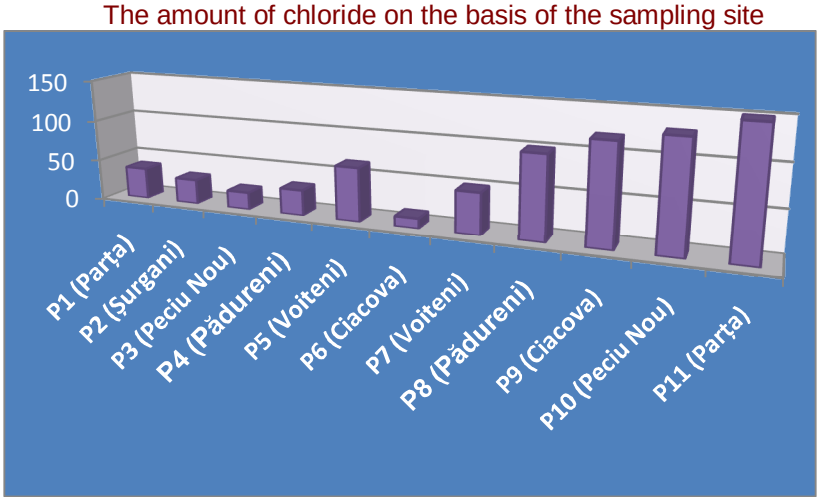


Figure 10. The stages of the dissolved oxygen test in samples

Figure 11. Lange SC1000 recording device for parameters in fish ponds

Recording quality parameters of samples from fish ponds is done daily, storing data in the event of their use and to detect malfunctions that may affect the welfare of fish in ponds (Table 3, Fig. 11).

According to the World Health Organization (1996) values obtained in the UK between the years 1974 to 1981 for chlorides in water were 14-42 ppm and

unpolluted areas with values between 1-10 ppm (77).

According to the U.S. Environmental Protection Agency maximum chlorides in water is 250 ppm. (78).

2.6. Conclusions

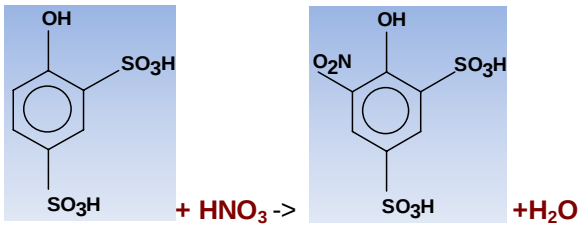
- Sampling at different weather conditions had an impact on the amount of chlorides by increasing its value in four sampling locations.
- The maximum of chlorides was recorded at Parța and was 150 ppm. The minimum amount of chlorine found in Ciacova with a value of 12 ppm.
- The amount of chlorine present in the water tested was within the permissible limits of legislation.

3. Determination of nitrates in water

3.1. Principle of method

It has been used a spectrophotometric method based on the property of the nitrate to react with the phenol-2,4-disulfonic acid with the formation of yellow nitroderivates, whose intensity is proportional to the concentration of nitrates in the sample (38).

Chemical formula:



3.2. Reagents

- phenol-disulfonic acid: was prepared from 1.2 g of crystalline phenol dissolved in 14.4 g concentrated H_2SO_4 (7.8 ml);
- ammonia, solution 25%;
- aluminum sulphate, 10%;
- nitrate standard solution: there were weighed 0.1631 g potassium nitrate and brought quantitatively into a volumetric flask of 100 ml (Figures 12-13).

Figure 12. Reagents used in determining

Figure 13. Analytical balances used for weighing reagents

3.3. Mode of action

- 1 ml H_2O (sample) was evaporated to dryness (Figures 14-15).
- 0,1 ml disulfonic acid
- stand for 15 min
- add 0.2 ml double distilled water
- adding 1 ml of 25% ammonium hydroxide NH_4OH
- read at 410 nm (Figures 14-19).

Figure 14. Evaporation samples in crucibles to dryness

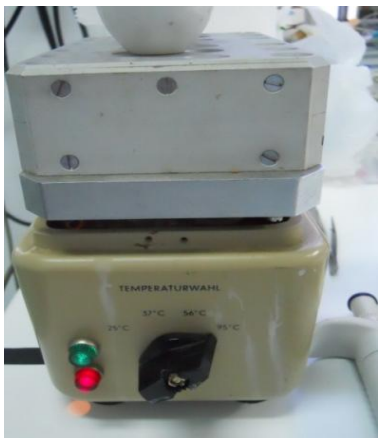


Figure 15. A device used for evaporation, set at 95^{0}C

Figure 16- (ensemble)

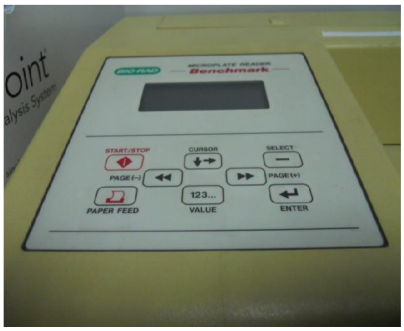


Figure 17. (panel detail**).** Benchmark spectrophotometer used to read samples

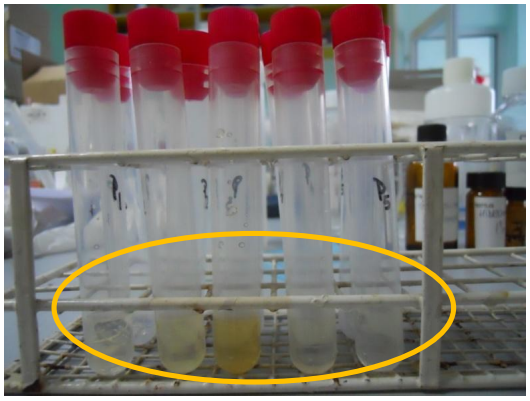


Figure 18. Change in yellow color according to the concentration of nitrates in samples

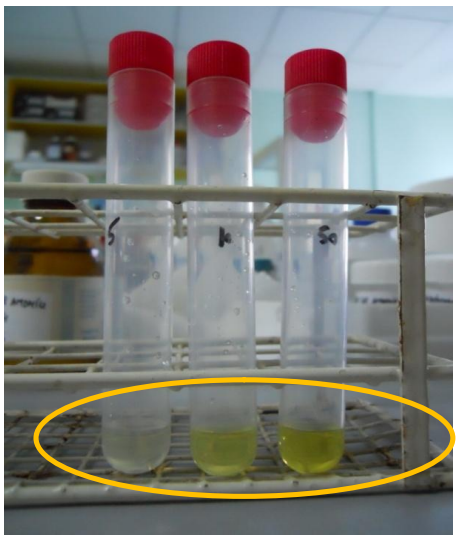


Figure 19. Nitrate standards (5-10-50)

3.4. Results and discussion

In order to achieve calibration curve were chosen three standards, whose absorbance was read in spectrophotometer with samples and the results shown in the table below (Tables 4-5, Figure 2).

Absorbance reading was performed for each sample / standard in double (Figures 3-4)

Table 4.
Absorbances recorded with spectrophotometer

Standards	Std (P1-P6)	Std (P1-P6)	Std (P7-P11)	Std (P7-P11)
5	0.160	0.166	0.136	0.137
10	0.265	0.270	0.854	0.799
50	1.879	1.961	2.257	2.109
P 1	0.363	0.371		
P 2	0.066	0.066		
P 3	0.221	0.222		
P 4	0.029	0.030		
P 5	0.023	0.026		
P 6	0.145	0.142		
P 7			0.246	0.257
P 8			0.043	0.043
P 9			0.078	0.083
P 10			0.035	0.036
P 11			0.166	0.170

Calculation of results

Nitrate concentration in the test sample was calculated according to the formula:

$$NO_3^- = mg/dm^3 = (C_x \times V)/V_p \quad \text{(formula1)}$$

Where:
C_x = nitrogen content of the photometry sample in µg/ml
V = flask volume in ml
V_p = the amount of water taken into consideration in ml

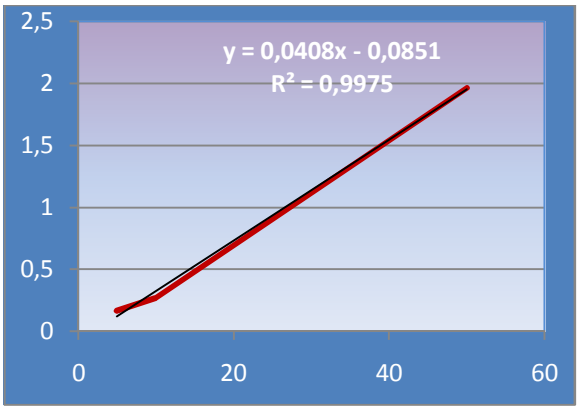
Table 5.

Nitrates of test samples

Samples	The amount of nitrate in the sample (mg/dm^3)	
P1	1,582	0,36
P2	1,872	1,872
P3	1,72	1,72
P4	1,907	1,907
P5	1,915	1,91
P6	1,795	1,797
P7	4,275	4,175
P8	4,075	3,975
P9	4.1	4
P10	4.05	3.95
P11	4.2	4.1

Graph 3.

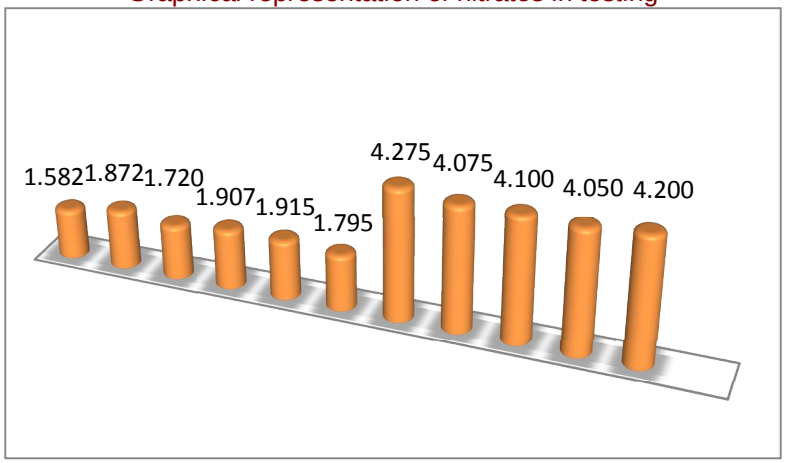
Calibration curve for P1-P6 standards



With Excel 2007, it was traced the calibration curve, y equation was calculated and the correlation coefficient (R^2).

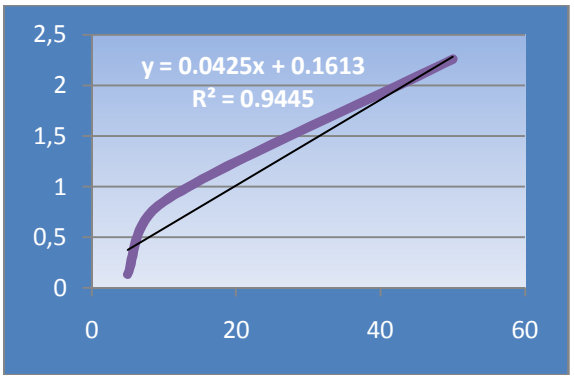
Graph 2.

Graphical representation of nitrates in testing



Graph 4.

Calibration curve for standards P7-P11



Under current legislation limits the amount of nitrates wants to be up in 50 mg / L (ppm) (Law no. 458 of 8 July 2002 on the quality of drinking water supplemented by Law no. 311/2004) (82).

According to the U.S. Environmental Protection Agency (1995) limit for nitrates in water is 10 ppm (85).

Researchers at the Center for Integrated Science (2003) conducted tests of water around a pig farm in Ithaca, NY, obtaining a value of between 100-150 ppm nitrate which exceeds the permissible value by legislation (86).

Schulz (2013) tested the nitrates in water obtaining values ranging from 0.02 to 300 ppm (57).

3.5. Conclusions

- The maximum level of nitrates was recorded for the sample taken from the village Voiteni (sample P7) with a value of 4275 ppm.

- Minimum amount of nitrate was recorded at the sample P1 and was 0.36 ppm.
- The amount of nitrate present in the waters tested was within the permissible limits of legislation.

4. Determination of phosphate in water

4.1. Principle of method

Phosphate anion reacted with ammonium molybdate in acidic medium and leads to ammonium phosphomolybdate:

$$H_3PO_4 + 12\ (NH_4)\ 2MoO_4 + 21HNO_3 \rightarrow (NH_4)3PO_4 \times 12MoO_3 + 21NH_4NO_3 + 2H_2O$$

Phospho-molybdate of ammonium form under the action of a reducing-stannous chloride, alkali metal sulfite, ascorbic acid, methanol, benzidine, amino acids – a blue complex:

$$[H_2PMo_{12}O_4]^- + 2\ \bar{e}\ [H_2PMo_{12}O_{40}]^{3-}$$

The blue complex is formed by molybdenum species in the oxidation stage Mo $^{5+}$ or in step Mo $^{6+}$:

$$[(Mo^{5+}O_2)_2]^{2+} \times [\ M_o^{6+}O_4]^{2-} \times H_2O$$

The color intensity is proportional to the phosphate concentration of the complex determined spectrophotometrically.

4.2. Consumables

- 96 well plate for reading at the spectrophotometer
- micropipettes of 200 µl and 1000 µl
- yellow and blue tips for micropipettes
- 3 or 5 ml vials, stoppered
- three liters of vegetable oil

4.3. Reagents

- 2.5 g Ammonium molybdate (fig. 20)
- 30 ml water
- 3.7 ml conc H_2SO_4
- cooling
- add 50 ml water in flask
- Sulphite (Na_2SO_3) 20% - was prepared fresh at the beginning of the workday
- Hydroquinone solution 1%
- Phosphate stock standard solution (0.1432 g KH_2PO_4 add 100 ml in flask)

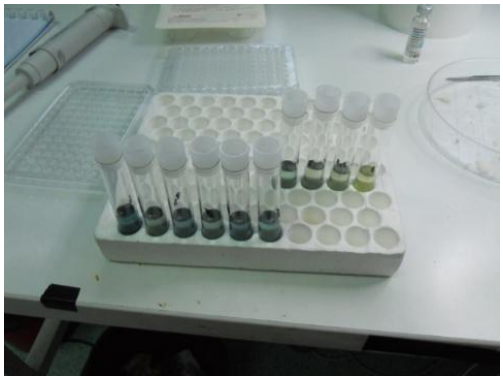
Figure 20. Reagents used in the determination of phosphate in water

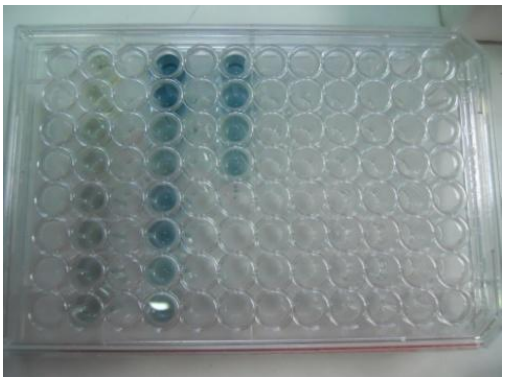
4.4. Mode of action

- 100 ml of sample was evaporated to dryness due to the low concentration of phosphates in the testing water, then there were introduced to the flask 2 ml of doubly distilled water, and the flask walls were washed for collecting deposits
- 0.4 ml Ammonium molybdate
- 0.2 ml Sulphite
- 0.2 ml hydroquinone
- Idle 30 minutes
- Reading at 655 nm

Evaporation of the water sample on oil bath was made at a temperature above 90^{0}C (Figures 21-22).

Figures 21-22. Evaporation of water sample on oil bath with Heidolph LABOROTA 4003 control unit





Figures 23-24. Colour reactions confirming the presence of phosphates in water samples and sample look before reading to the spectrophotometer

Samples were placed in plates with wells at an amount of 250 µl per well and were kept in a dark place during the 30 minutes before reading because phosphorus sensitivity to light (Figures 23-24).

In order to achieve calibration curve were chosen three standards, 1, 2.5 and 5 ppm, which were read together with the samples (Table 6).

Table 6.

Absorbances of test samples

Standards	Std (P1-P6)	Std (P1-P6)	Std (P7-P11)	Std (P7-P11)
1	0.077	0.077	0.033	0.034
2.5	0.161	0.165	0.053	0.050
5	0.266	0.265	0.089	0.083
P 1	0.675	0.686		
P 2	0.288	0.282		
P 3	0.411	0.426		
P 4	0.308	0.302		
P 5	0.451	0.460		
P 6	0.298	0.297		
P 7			0.299	0.296
P 8			0.326	0.347
P 9			1.000	1.063
P 10			0.483	0.460
P 11			0.171	0.179

4.5. The results of testing phosphates

Calculation of results:

Calculation of phosphorus in water was based on the following formula:

$$PO_4^{3-} (mg/dm^3) = (C_x \, xV) / V_1 \quad \text{(formula 2)}$$

Unde:

C_x = phosphate ion concentration in the spectrophotometric sample (µg/ml)

V = flask volume (ml)

V_1 = the amount of water taken into consideration

Absorbance value read reported to the calibration curve for finding the phosphate content of the sample (Table 7, Figure 7).

Table 7.

The amount of phosphates specified in the test

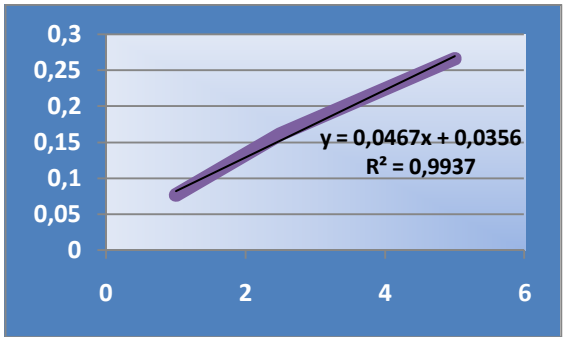
Samples	The amount of phosphate in the sample (mg/dm^3)	
P1	0.0351	0.0356
P2	0.0170	0.0167
P3	0.0227	0.0230
P4	0.0179	0.0170
P5	0.0246	0.0250
P6	0.0175	0.0174
P7	0.0055	0.0055
P8	0.0057	0.0055
P9	0.0080	0.0082
P10	0.0062	0.0060
P11	0.0050	0.0055

The calibration curve was obtained by correlating the concentrations in Excel 2007 based on absorbances read and then traced chart for calibration right (Figures 5-6).

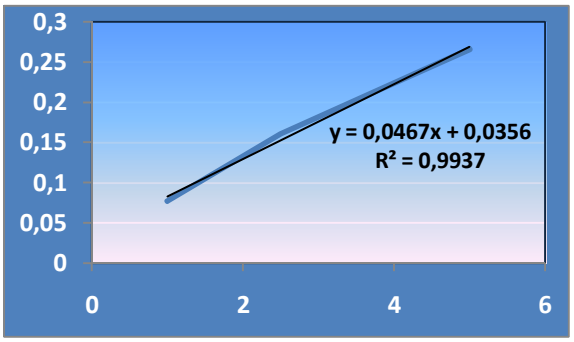
Phosphate limits were determined by the correlation with the allowed values and were to 0.1 ppm (81).

Graph 5.

The calibration curve of the standard P1-P6



Graph 6.
The calibration curve of the standards P7-P11



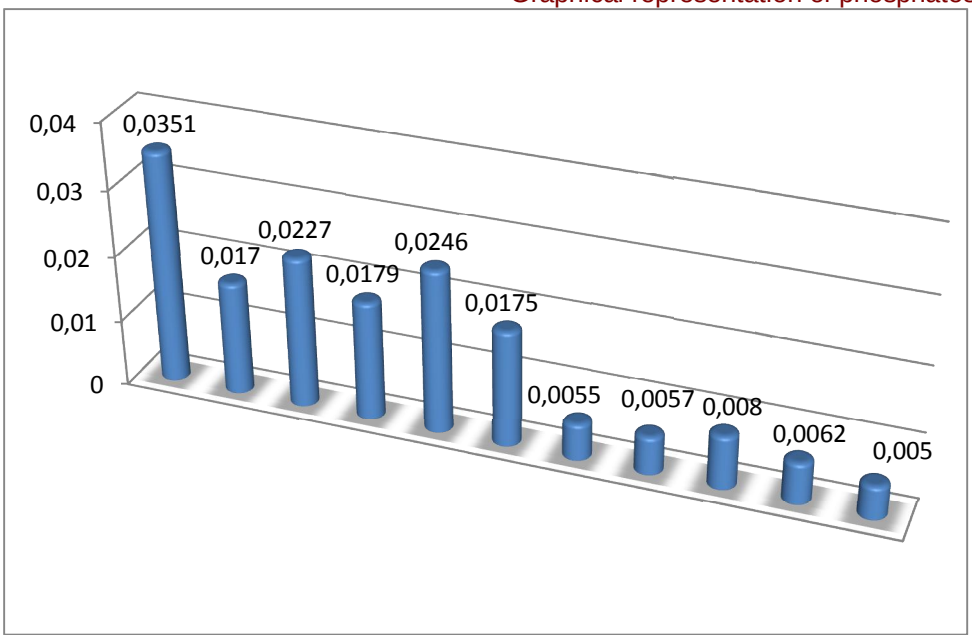
According to the U.S. Environmental Protection Agency (1986) limits of the amount of phosphates in lakes is 0.05 ppm and surface water between 0.01-0.03 ppm (76).

In natural waters limit of Indianapolis Center for Environmental Sciences phosphates is 0.02 ppm (79).

4.6. Conclusions

- Phosphates maximum values were recorded for P1 sample and the was 0.0356 ppm.
- The minimum amount of phosphate was detected for sample P11 with a value of 0.005 ppm.
- The amount of phosphate present in the waters tested was within the permissible limits of legislation.

Graph 7.
Graphical representation of phosphates in testing



5.5. Making determinations of potentially polluting compounds by GC-MS

5.1. Materials

- flask with stopcock (fig. 25).
- Metal support for flask
- Cylinder
- Glass funnel
- Nitrile gloves
- Brown glass bottles with screw cap

Figure 25. The balloon used in the extraction

5.2. Reagents

There were used substances from Sigma-Aldrich and Fluka Chemika (Figures 26-27).

Figures 26-27. Bottles with dichloromethane 99.9% (liquid solution), and anhydrous sodium sulfate (powdered)

5.3. Sample preparation for GC-MS

In order to achieve the extract it was used one liter of sample water placed over with 20 ml of dichloromethane, an attempt was made by stirring the flask homogeneous. It was allowed to stand to separate visible the dichloromethane sample until a clear separation of the water-oil (Fig. 28).

Dichloromethane was collected in a glass container without allowing penetration of any amounts of water.

In the presence of a small amount of the sample in the collection container, and blisters of air were observed on the surface there is necessary to treat the extract with anhydrous sodium sulfate.

The extracts were evaporated to dryness (Fig. 29) and were frozen at -20^{0}C until the time of determination by GC-MS.

Figure 28. Separarea straturilor, probă de apă – diclormetan

Before the determination, the extracts were re-suspended in 100 µl of dichloromethane and then injected in the GC-MS in an amount of 1 µl.

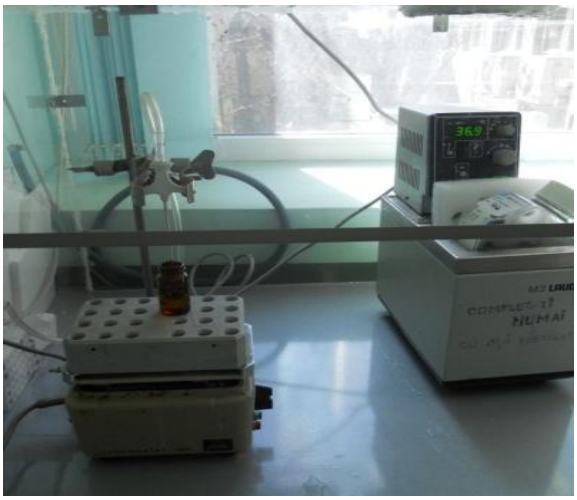


Figure 29. Evaporation of the extracts to dryness under a hood controlled process with oxygen to speed up the introduction and maintenance of evaporation of the container in a heat source

5.4. GC-MS method of determination

Samples analyzes by GC-MS were performed at the Institute of Immune physiology at Timișoara County Hospital.

Water samples were kept refrigerated (4^{0}C) from harvest to the time of analysis.

Determination by GC-MS was performed by using Hewlett-Packard gas chromatograph model 6890 (Fig. 30) using the mass spectrometer detector Hewlett-Packard model 5973 (fig. 31).

Separation was achieved using column ZB (Zebron) - 5 MS, 30 m in length, with the inner diameter 0.25 mm and film thickness of 0.25 µm.

In the determination were used: He with a constant flow of 1 ml / minute at a temperature of 250^{0}C injector type splitless mode.

Program temperature and time of arrival at a particular temperature were recorded as being from 50^{0}C to 300^{0}C with 6^{0}C increase per minute and was maintained final for 1 min at 300^{0}C.

Injected sample volume was 1 µl. Ionization detector used was EI (electron ionization) type.Transfer line was 230^{0}C MS source to and 150^{0}C MS Quad. Scanning took as parameters: solvent delay of 7 minutes, the 50-550 atomic mass units. The database containing the spectra library was NISTO2 (Fig. 32). Printer attached to the devices was produced by the same company Hewlett-Packard Laserjet 4000 (fig. 33).

Interpretation of results was done with MSD ChemStation Agilent Technologies D.02.00.275 product.

Figure 30. Gas chromatograph Hewlett-Packard model 6890

Figure 31. Hewlett-Packard mass spectrometer model 5973

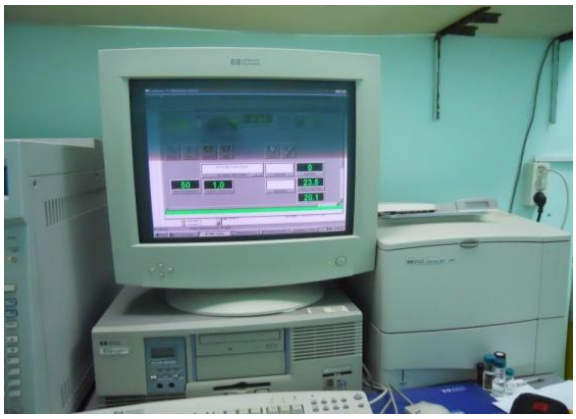


Figure 32. Related software for recording computer data from GC-MS

Figure 33. A data output device Hewlett-Packard LaserJet 4000 coupled to GC-MS

5.5. The results of analysis by GC-MS

GC-MS analysis revealed the presence of substances in quantity as water samples can be compared with limits permissible under applicable law (Table 8).

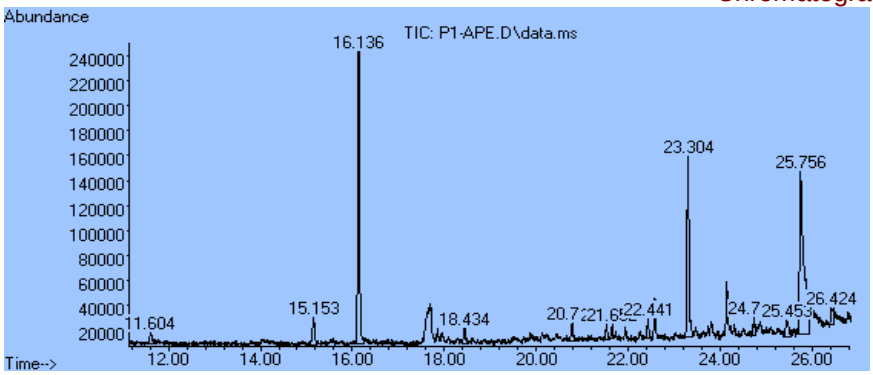
Table 8.

Substances detected by GC-MS for sample 7

Crt. no.	Retention time	Common name of the group of the detected substance	Chemical name	AREA%
1	11.6	Phenols	Phenol, m-tert-butyl-	1.42
2	15.15	Halogenated	2,5-cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)	3.51
3	16.13	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	30.65
4	18.44	Polycyclic aromatic hydrocarbons	Benzophenone	1.67
5	20.78	Alkali-saturated acyclic hydrocarbons	2,3,5,6-detetrahydrocyclohexanone, 2,6-di-t-butyl-4-hydroxymethylene-	1.78
6	21.52	Saturated hydrocarbons	5-octadecene	1.46
7	21.65	Aromatic hydrocarbons	Benzene,1,1'-(2,2-dichloroethylidene)bis[4-ethyl	1.05
8	22.44	Halogenated	2,5-di-tert-butyl-1,4-benzoquinone	1.83
9	23.30	Halogenated	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	16.56
10	24.73	Halogenated	9,10-anthracenedione, 1-ethyl-	1.36
11	25.45	Halogenated	10,18-bisnorabieta-8,11,13-triene	2.16
12	25.75	Heterocyclic compounds	1,3-dicyclohexylurea	34.53
13	26.41	Phenols (commercial name:Triclosan)	polychloro phenoxy phenol	1.95

Graph 8.

Chromatogram of sample 7



In the case of chromatogram for sample P7 (Graph 8) have been registered high values of phenols at an area of 30.65%, with a retention time of 16.13 and for heterocyclic compounds 34.53% area and the retention time of 25.75.

The lowest values registered by GC-MS for sample P7 were for aromatic hydrocarbons of 1.05% area and a retention time of 21.65.

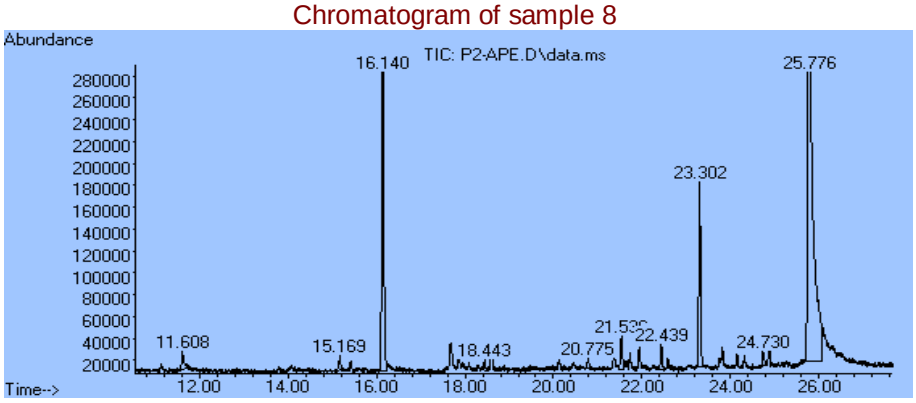
Table 9.

Substances detected by GC-MS for sample 8

Crt. no.	Retention time	Common name of the group of the detected substance	Chemical name	AREA%
1	11.6	Phenols	Phenol, m-tert-butyl-	1.23
2	15.15	Halogenated	2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.71
3	16.13	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	30.98
4	18.44	Polycyclic aromatic hydrocarbons	Benzophenone	0.41
5	20.78	Alkali -acyclic saturated hydrocarbons	2,3,5,6-detetrahydrocyclohexanone, 2,6-di-t-butyl-4-hydroxymethylene-	0.40
6	21.52	Saturated hydrocarbons	5-octadecene	1.58
7	22.44	Halogenated	2,5-di-tert-butyl-1,4-benzoquinone	1.05
8	23.30	Halogenated	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	7.99
9	24.73	Halogenated	9,10-anthracenedione, 1-ethyl-	0.78
10	25.75	Heterocyclic compounds	1,3-dicyclohexylurea	54.83

Graph 9.

Chromatogram of sample 8



In the case of chromatogram for sample P8 (Graph 9) have been registered high values of heterocyclic compounds at an area of 54.83%, with a retention time of 25.75 and for phenols at 30.98% area and the retention time of 16.13.

The lowest values registered by GC-MS for sample P8 (Table 9) were for alkali-acyclic saturated hydrocarbon of 0.4% area and a retention time of 20.78.

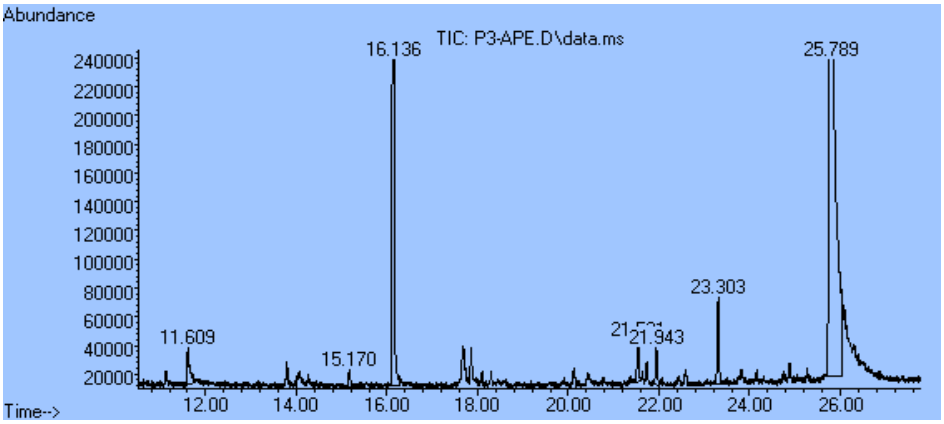
Table 10.

Substances detected by GC-MS for sample 9

Crt. no.	Retention time	Common name of the group of the detected substance	Chemical name	AREA%
1	11.6	Phenols	Phenol, m-tert-butyl-	1.801
2	15.15	Halogenated	2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.552
3	16.13	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	30.702
4	21.53	Alkenes	9-tricosene	1.35
5	21.94	Saturated aliphatic hydrocarbons	Cyclotetracosane	1.15
6	23.30	Halogenated	2,5-cyclohexadien-1-one, 2,6-bis (1,1-dimethylethyl)-4-ethylidene-	2.64
7	25.75	Heterocyclic compounds	1,3-dicyclohexylurea	61.78

Graph 10.

Chromatogram of sample 9



In the case of chromatogram for sample P9 (Graph 10) have been registered high values of heterocyclic compounds at an area of 61.78%, with a retention time of 25.75 and for phenols at 30.70% area and the retention time of 16.13 (Table 10).

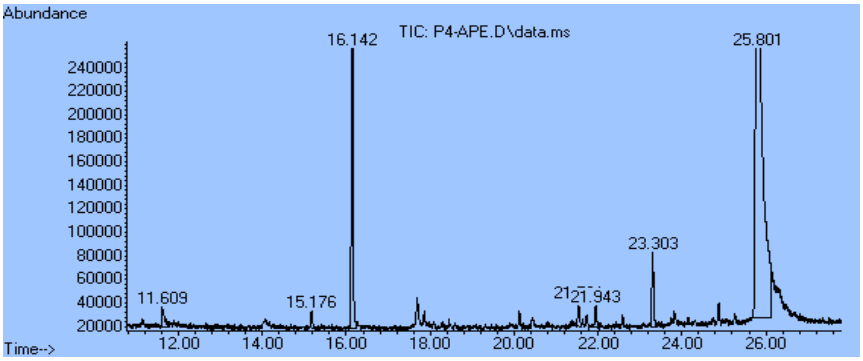
The lowest values registered by GC-MS for sample P9 were for halogenated of 0.552% area and a retention time of 15.15.

Table 11.

Substances detected by GC-MS for sample 10

Crt. no.	Retention time	Common name of the group of the detected substance	Chemical name	AREA%
1	11.6	Phenols	Phenol, m-tert-butyl-	1.25
2	15.15	Halogenated	2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.57
3	16.13	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	19.71
4	21.53	Alkenes	9-tricosene	0.95
5	21.94	Saturated aliphatic hydrocarbons	Cyclotetracosane	0.79
6	23.30	Halogenated	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	2.74
7	25.75	Heterocyclic compounds	1,3-dicyclohexylurea	73.95

Graph 11.
Chromatogram of sample 10



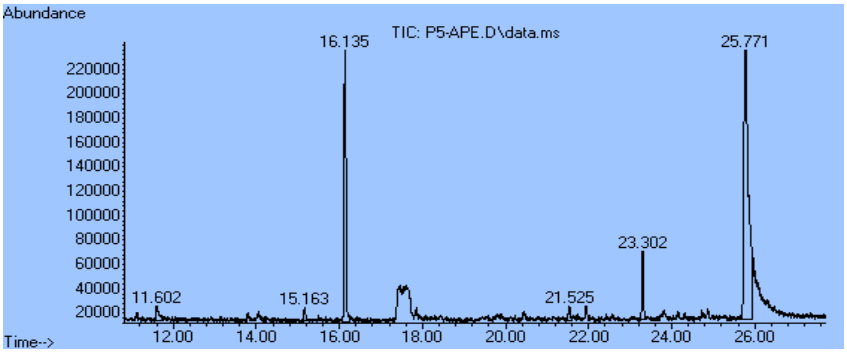
In the case of chromatogram for sample P10 (Graph 11) have been registered high values of heterocyclic compounds at an area of 73.95%, with a retention time of 25.75 and for phenols at 19.71% area and the retention time of 16.13 (Table 11).

The lowest values registered by GC-MS for sample P10 were for halogenated of 0.57% area and a retention time of 15.15.

Table 12.
Substances detected by GC-MS for sample 11

Crt. no.	Retention time	Common name of the group of the detected substance	Chemical name	AREA%
1	11.6	Phenols	Phenol, m-tert-butyl-	1.93
2	15.16	Halogenated	2,5-cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl)-	1.08
3	16.13	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	26.89
4	21.52	Alkenes	9-tricosene	1.39
5	23.30	Halogenated	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	5.22
6	25.77	Heterocyclic compounds	1,3-dicyclohexylurea	63.47

Graph 12.
Chromatogram of sample 11



In the case of chromatogram for sample P11 (Graph 12) have been registered high values of heterocyclic compounds at an area of 63.47%, with a retention time of 25.77 and for phenols at 26.89% area and the retention time of 16.13 (Table 12).

The lowest values registered by GC-MS for sample P11 were for halogenated of 1.08% area and a retention time of 15.16.

For all samples analyzed by GC-MS, detection of phenols had a retention time of 16.13, heterocyclic compounds retention time was the same for samples P7, P8, P9, P10, and in the samples P9 and P10 it was detected a retention time identical for halogenated.

These results draw the warning signals because phenol is toxic protoplasmic causing hypothermia and paralysis of the vasomotor center, thus showing increased action on the central nervous system.

It also may be absorbed through the skin, respiratory and digestive mucous membranes.The action depends on the concentration of phenols, such as diluents may cause congestion and ulceration of the skin and concentrated are corrosive to skin and mucous membranes (75).

Maximum permissible limit for phenols in waste water bound for rivers is 30 ppm according to HG 188 / 20.03.2002 approving the rules on the conditions for discharge of wastewater into the aquatic environment, as amended by Government Decision 352/11.05.2005 (84).

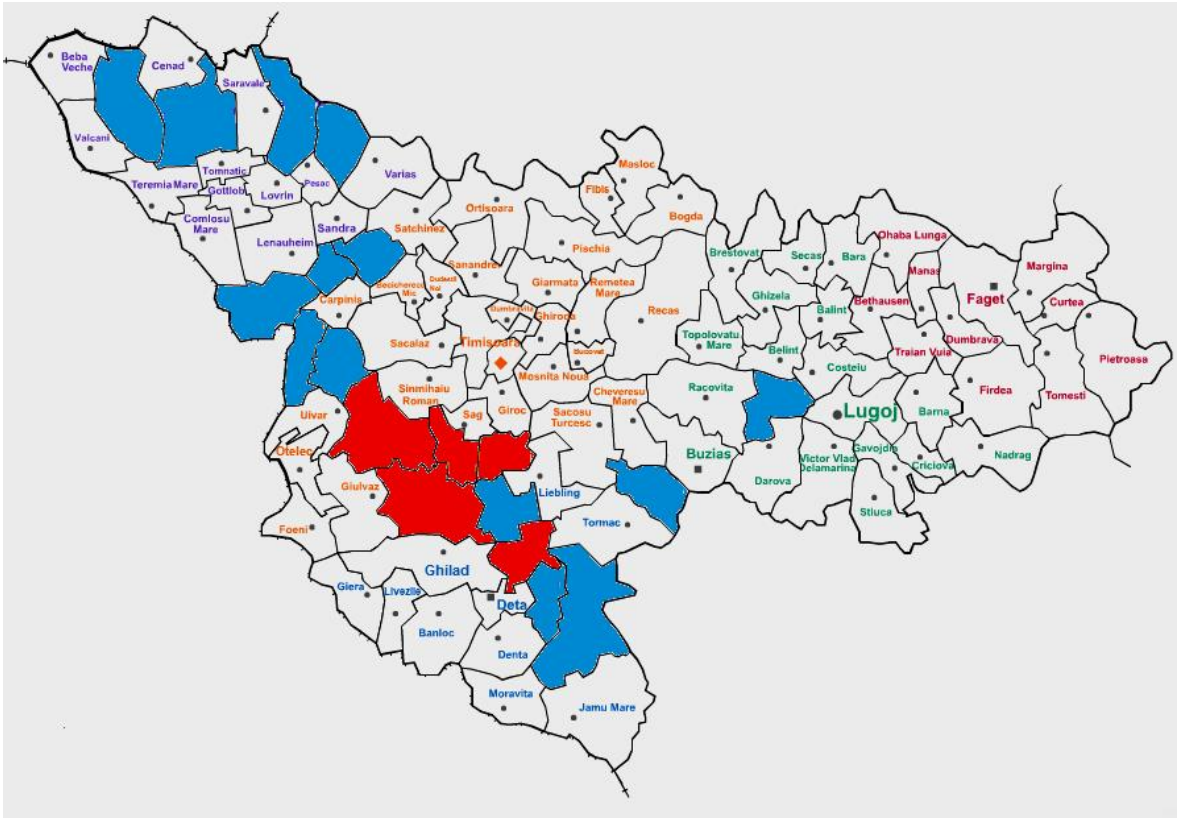


Figure 34. Pollution vulnerable areas in Timiș County (80, 83).

Where:

Blue - Vulnerable localities in the county

Red - Localities from where the water samples were taken that are found to be vulnerable zones

5.6. Conclusions

- Determination by GC-MS showed a higher proportion of phenol in the water samples suspended in dichloromethane.
- The amount of phenols present in the waters tested was within the permissible limits of legislation.

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