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# EFFICIENT CORROSION INHIBITORS BY RECOVERY OF PHARMACEUTICAL WASTE

**Ioana MAIOR [1], Anca COJOCARU [1], Gabriela-Elena BADEA [2], Ioana-Maria NICOLA [1], Ioana-Alina CIOBOTARU [1] , Adina BARANGA [1]**

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***Abstract****. The main objective of the present paper is the preliminary investigation and development of new applications for expired drugs, in contrast to the current regulations regarding the controlled destruction of pharmaceutical waste by different methods. The new applications studied are based on the anticorrosive inhibitory properties of the active compounds from different drugs and even of the compositions as such, on metallic materials in aggressive environments with acid pH. In this paper, the possibility to recover pharmaceutical waste achieved from expired drugs, paracetamol (PA) and carbocysteine (CS), in order to use it as corrosion inhibitors for carbon steel in acid solutions has been investigated. 1N HCl aqueous solution has been used as corrosive medium in the experimental studies. Electrochemical behavior of PA and CS and its inhibitor efficiency in test solutions have been examined by open circuit potential and gravimetric methods.*

***Keywords****: pharmaceutical waste, paracetamol, carbocysteine, acid solution, corrosion efficient inhibitor*

## 1.INTRODUCTION

Lately, the use of expired drugs as corrosion inhibitors for different metals and alloys represents an important challenge in terms of recovering the active compounds from pharmaceutical drugs existing in increased amounts, both in households and pharmacies, hospitals, etc. The properties that recommend the use of active substances from expired drugs in corrosion prevention are high molecular weight and size, the presence of hetero atoms (nitrogen, sulphur, oxygen etc. due to the pair of non-participating electrons) and π bonds in their structures.[1–5] Also, most of these compounds are highly soluble in aqueous solutions and stable in different aggressive medium.[2–7] It is necessary to consider the fact that in the pharmaceutical dosage different types of drugs contain several active compounds, but also a number of excipients intended to ensure stability and bioavailability. These excipients can be separated by a simple operation, decantation or filtration, due to the differences of solubility with respect to the active substance. In studying the inhibitory effect of drugs, it is necessary to consider the possible chemical reactions of the active substance in the corrosive environment. In addition, synergistic effects must be taken into account when drugs contain more than one active substance.

The efficiency of an organic compound as an inhibitor is mainly dependent on its ability to be adsorbed on the metal surface, a process consisting of replacing the water molecules on the surface immersed in the aggressive solution. The adsorption of these compounds is influenced by the electronic structure of the inhibitor molecules, the steric hindrance factor, the aromaticity and the electron density at the donor

surface, the presence of functional groups and the molecular mass. The metal corrosion-inhibiting action of some drugs has been attributed to blocking of the substrate surface by forming insoluble complexes at the metal–aggressive environment interface.

It has been reported that after the expiration date, for more than 90% of the drugs, the active constituents do not degrade and maintain their stability for a long period.[1, 3, 4] Based on presented considerations, expired drugs are safe to be used, and because of their negligible negative impact on the environment, these commercial pharmaceutical formulas seem to be potential candidates to replace toxic chemical corrosion inhibitors.[1, 8–10] Moreover, due to the possibility of recovery of such wastes, the expired drugs were included in *green corrosion inhibitors* family.

However, most pharmaceutical drugs are much more expensive than the organic inhibitors currently used in the industry. Thus, the use of a drug within the term of validity as a corrosion inhibitor is not economically viable. Therefore, it is considered useful to investigate the corrosion inhibition properties of expired drugs that can no longer be used for medical purposes.[4, 5, 11–14] In the context of an increasing amount of expired drugs, their use as corrosion inhibitors can not only reduce environmental pollution, but can also lead to a reduction of disposal and degradation costs of these pharmaceutical wastes. The use of expired paracetamol as a corrosion inhibitor for metals and alloys in acidic medium has become a topic of great interest only in recent years, but no complete experimental studies have been performed.[4, 5, 15–20]

In this paper, the possibility to recover pharmaceutical waste achieved from expired drugs in syrup form, paracetamol (PA) and carbocysteine (CS), in order to use it as corrosion inhibitors for carbon steel in acid solutions has been investigated. 1N HCl aqueous solution has been used as corrosive medium in the experimental studies. Electrochemical behavior of PA and CS and its inhibitor efficiency in test solutions have been examined by open circuit potential and gravimetric methods.

## 2.EXPERIMENTAL PART

### 2.1.Materials

In order to study the corrosion behavior of carbon steel in acidic solutions and the inhibitory effect of the expired drugs – paracetamol and carbocysteine in commercial syrup form – different concentrations of 1.2% and 2.4% respectively were used. The corrosive medium was prepared from hydrochloric acid (Merck, 37% concentration). The working electrode used was a rectangle with an active surface of 14.56 $cm^2$, cut from a sample of carbon steel OL 52 with the elemental composition presented in Table 1.

***Table 1.*** *Elemental composition of carbon steel OL 52 samples*

| Element | Fe | C | Si | Mn | P | S | Al | Cu |
|---|---|---|---|---|---|---|---|---|
| Weight, % | 98.58 | 0.196 | 0.034 | 0.896 | 0.013 | 0.037 | 0.023 | 0.125 |

**2.2.Experimental investigation methods**

The electrochemical behavior of the expired drugs in acidic environment was studied by open-circuit potential measurement (OCP) method and also by gravimetric method, electrochemical techniques necessary to highlight the corrosion inhibition properties of PA and CS on the corrosion process of carbon steel. The experimental electrochemical determinations were performed in a typical 50 mL glass cell, thermostated, being equipped with three electrodes and connected to a VoltaLab 40 PGZ potentiostat / galvanostat. The potentiostat was connected by a platinum sieve auxiliary electrode with a large active surface, a working electrode made of carbon steel with a surface of 0.5 $cm^2$ and the saturated Ag/AgCl as reference electrode. All potentials were reported at this reference electrode ($E_{Ag/AgCl}$ = +0,199 V / NHE). The working electrode potential was stabilized for 15 minutes before each measurement.

The electrochemical behavior of the expired drugs – commercial paracetamol and carbocysteine in syrup form, respectively – was studied by gravimetric method, performed to highlight the properties of corrosion inhibition by determining the corrosion rate and the inhibition efficiency. The experimental electrochemical determinations were performed in glass Berzelius glasses, maintained at a constant temperature of 25°C, with a volume of 400 mL, being equipped with three steel samples. The surface of the working electrode was polished before each experiment with abrasive paper, cleaned in an ultrasonic bath and rinsed with distilled water, dried and then weighed. After the immersion time in the acid solution expired, corrosion products were removed from the steel sample surface by buffering with ammonium citrate solution, rinsing with distilled water and drying, then the electrodes were again weighed.

**3. RESULTS AND DISCUSSIONS**

The electrochemical oxidation of paracetamol in acid solution is a quasi-reversible process, which takes place in two stages, according to the mechanism presented by Nematollahi, D. et all [13] and resumed by Duca, D.A. et al [5]. In the first stage, by releasing two electrons and two protons, paracetamol is oxidized to N-acetyl-p-benzoquinonimine, and in the second stage the hydrolysis of this compound takes place.

The effect of adding expired paracetamol to the acidic solution leads to a decrease in the chlorine evolution reaction and a shift of the OER potential (oxygen evolution reaction) to more electropositive values. HER (hydrogen evolution reaction) is inhibited due to the adsorption of paracetamol molecules on the working electrode surface.

**3.1. The open circuit potential method**

In order to elucidate the corrosion behavior of OL 52 samples in acid solutions, OCP measurements were performed, compared to different concentrations of paracetamol and carbocysteine.

Potential-time curves *(ε–t)* were recorded over a period of 15 minutes in the case of two different concentrations of the investigated drugs, for carbon steel samples in acid test solutions. Figure 1 shows the *(ε–t)* curves recorded on the test electrode, OL52, in 1 $mol \cdot L^{-1}$ HCl solution, in the absence and presence of paracetamol and carbocysteine.

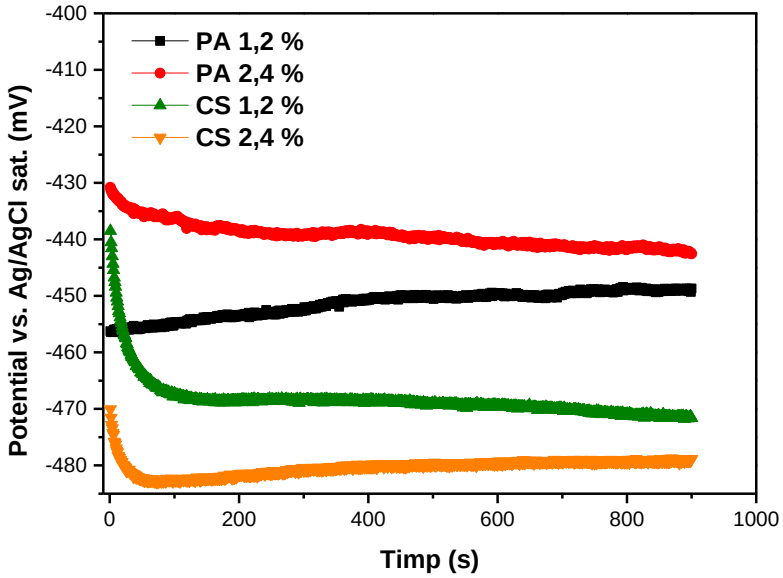


***Figure 1.*** *Variation of open circuit potential as a function of exposure time in 1N HCl solution at different concentrations of PA and CS*

Analyzing the graphical results, a common feature of carbon steel electrooxidation in both tested inhibitors is the rapid stabilization of the potential value after about 5 minutes, probably due to the formation and growth of a passive oxide film, evenly distributed on the metal surface.[14]

It is observed from Figure 1 that, in the case of paracetamol, the open circuit potential evolves towards more electropositive values, higher by 30-40 mV compared to the corresponding potential of fluidol (carbocysteine). In the case of fluidol, a sudden decrease in potential can be observed in the first minutes after exposure of the steel sample to the acid medium, followed by a tendency to stabilize at a constant value.

The increasing potential values for oxidized samples in the acid medium with paracetamol or carbocysteine are due to the formation of a protective film of organic molecules on the active surface of the test electrode, which prevents or decreases the corrosion rate of carbon steel.

### 3.2. Evaluation of corrosion rate by gravimetric method

Furthermore, the corrosion behavior in acid medium (1N HCl) of 15 carbon steel plates, with an active surface area of 14.56 $cm^2$, was tested, as well as the inhibition activity of two expired drugs, namely: Paracetamol (PA) syrup of 120 mg / 5ml concentration, and Fluidol (CS) syrup of 250 mg / 5ml concentration.

Prior to immersion in the solution, the plates were polished with abrasive paper, cleaned in an ultrasonic bath and rinsed with distilled water. The corrosive medium investigated was an aqueous solution of 1N HCl. The weight loss was determined after 24 h, 7 days, and 14 days, respectively, for the carbon steel plates immersed in acid solutions, to which was added an inhibitor content of different concentrations: paracetamol syrup 1.2% and 2.4%; carbocysteine syrup 1.2% and 2.4%.

The appearance of steel plates are shown in figures 2-4.

The weight loss of steel samples was calculated by means of the following relation:

$$\Delta m = m_0 - m_1, \text{ g} \quad (1)$$

where:

$m_0$ – the initial mass of the sample in g; $m_1$ – the final mass of the sample in g.

The corrosion rate of carbon steel ($v_{corr}$) was determined using the relation:

$$v_{corr} = \frac{\Delta m}{S \cdot t}, \text{ g/m}^2\cdot\text{h} \quad (2)$$

where:

Δm – the weight loss in the presence and the absence of inhibitor, respectively; S – the total surface area exposed to aggresive solution, in $m^2$; t – the exposure time, in hours.

The inhibition efficiency (IE) was determined with equation (3):

$$IE\ (\%) = \left(\frac{v_{corr}^{o} - v_{corr}^{inh}}{i_{corr}^{o}}\right) \cdot 100 \quad (3)$$

where

$v_{corr}^{o}$ and $v_{corr}^{inh}$ are the corrosion rates in the absence and the presence of inhibitor, respectively.

From the experimental data, the corrosion rates and the inhibition efficiency were calculated for each plate, results presented in Table 2.

The experimental and calculated data presented in Table 2 show that inhibitor efficiency reaches appreciable values for both PA and CS concentrations.

As expected, the weight loss of carbon steel samples are decreasing in the presence of expired drug with increase of the concentration.

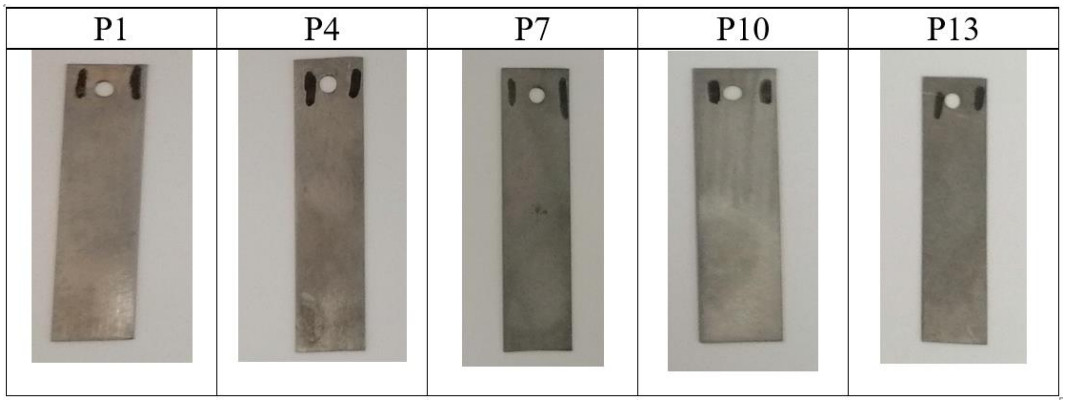


***Figure 2.*** *Appearance of steel plates after 24 hours immersion in 1N HCl*

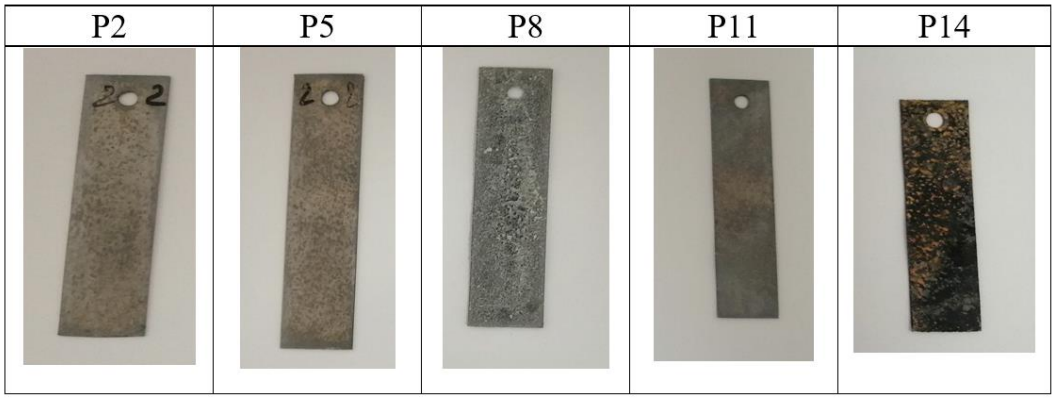


***Figure 3.*** *Appearance of steel plates after 7 days immersion in 1N HCl*

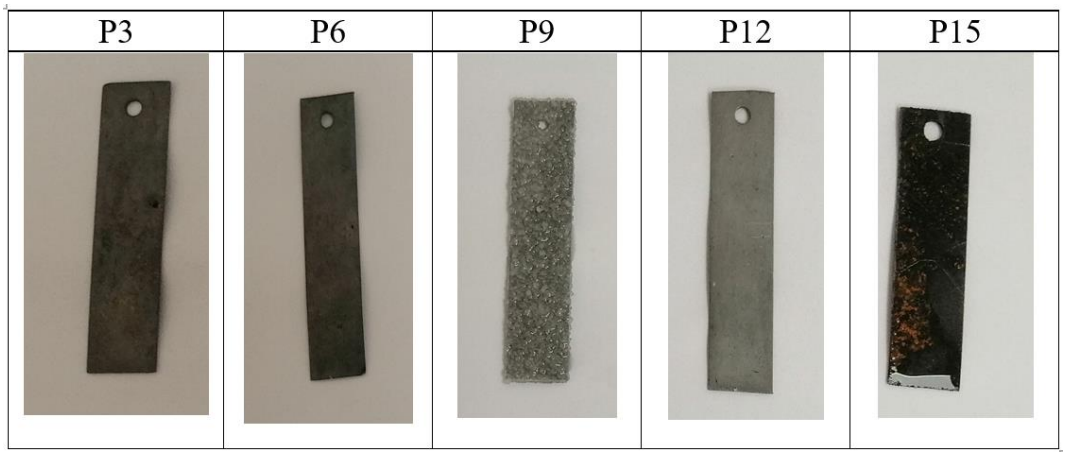


***Figure 4.*** *Appearance of steel plates after 14 days immersion in 1N HCl*

***Table 2.*** *Parameters calculated by gravimetric method expressed as a function of immersion time in 1N HCl solution at different concentrations of PA and CS*

| Sample | Corrosive medium | Exposure time, h | Weight loss Δm, g | Corrosion rate $v_{corr}$, $g \cdot m^{-2} \cdot h^{-1}$ | Inhibition efficiency IE, % |
|---|---|---|---|---|---|
| $P_1$ | HCl 1N + PA 1.2% | 24 | 0.0622 | 1.78 | 92 |
| $P_2$ | | 168 | 0.3565 | 1.46 | 80 |
| $P_3$ | | 336 | 1.518 | 3.11 | 20 |
| $P_4$ | HCl 1N + PA 2.4% | 24 | 0.0088 | 0.25 | 98 |
| $P_5$ | | 168 | 0.3059 | 1.25 | 83 |
| $P_6$ | | 336 | 0.6111 | 1.24 | 68 |
| $P_7$ | HCl 1N + CS 1.2% | 24 | 0.0829 | 2.38 | 89 |
| $P_8$ | | 168 | 0.4528 | 1.85 | 75 |
| $P_9$ | | 336 | 1.5457 | 3.17 | 19 |
| $P_{10}$ | HCl 1N + CS 2.4% | 24 | 0.0092 | 0.26 | 98 |
| $P_{11}$ | | 168 | 0.3345 | 1.37 | 82 |
| $P_{12}$ | | 336 | 0.7523 | 1.54 | 61 |
| $P_{13}$ | HCl 1N | 24 | 0.8047 | 23.12 | - |
| $P_{14}$ | | 168 | 1.8107 | 7.43 | - |
| $P_{15}$ | | 336 | 1.917 | 3.93 | - |

Both tested inhibitors show similar values of inhibition efficiency, between 89 and 98%, as well as a progressive decrease over time of these values, most likely due to the decrease of the inhibitor concentration in the aggressive environment.

Regarding the initial concentration of these tested inhibitors, higher values of corrosion rates were found at the initial concentration of 2.4% compared to 1.2%, having similar values for both inhibitors investigated.

However, a more efficient behavior of paracetamol than that of fluidol can be found for both tested concentrations, although the calculated values of the corrosion rates are very close.

The presented experimental studies prove excellent inhibitory properties

of expired drugs investigated for the corrosion process of OL 52 carbon steel in 1N HCl.

Maximum inhibition efficiency values of 98% were obtained for both inhibitors, after 24 hours of immersion, respectively 61-68% after 2 weeks of immersion in 1N HCl.

In aqueous acid solutions, the adsorption of expired drug molecules can be considered as a quasi-substitution process between the drug in the aqueous phase and the water molecules on the metal surface.[4, 12, 13]

## 4.CONCLUSIONS

The inhibitor adsorbed on the metal surface blocks either the anodic reaction, the cathodic reaction, or even both. It can cause changes in the electrical double layer, by reducing the reactivity of the metal, due to the participation in the partial electrode reaction and the formation of a physical barrier by increasing the ohmic resistance of an inhibitor film at the metal-electrolyte interface. The adsorbed inhibitor may not cover the entire surface of the metal, but may fill in the electrochemically active sites and therefore the extent of the anodic, cathodic, or both reactions is reduced. The corrosion rate will decrease proportional to the expansion on the electrochemically active areas that are blocked by the adsorbed inhibitor.

Anodic dissolution of metals is assumed to be a stepwise reaction, with intermediates adsorbed on the metal surface. In the anodic dissolution of iron or steel, the adsorbed intermediate is considered to be FeOH and, on the addition of an organic inhibitor, a complex is formed, which is adsorbed on the metal surface. This surface complex modifies the reaction mechanism, causing an increase in the anodic Tafel slope.

By comparing the corrosion rates, it can be seen that both types of drugs in the form of syrup, paracetamol PA and Fluidol CS, respectively, have corrosion inhibition properties for carbon steel samples exposed to aggressive acidic solution of 1N HCl.

In the acid solution, the steel is oxidized in two stages, according to a quasi-reversible process, the oxidation products formed being adsorbed on the active surface of the samples, thus contributing to the increase of the inhibitory efficiency.

The results show that the PA and CS molecules or their oxidation products form by adsorption a barrier layer on the surface of the OL52 sample, thus inhibiting the corrosion process.

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# ANTIMICROBIAL EFFECT OF GENTAMICIN INCORPORATED IN HYDROXIAPATITE COATING ON 316L STAINLES STEEL

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## Abstract

*This study aims on morphological, antibacterial and drug release characterization of a coating based on hydroxyapatite (HA)/with or without gentamicin on 316L stainless steel, in physiological environment. Hybrid materials structure was identified by Fourier transformed infrared spectroscopy (FTIR) and their surface analysis by scanning electron microscopy (SEM).*

*Gentamicin release were studied using a UV-Vis method. Antibacterial properties of coatings were determined by contact method using using agar disc diffusion method for two bacterial strains: S. aureus and E. coli.*

*Antibacterial effect of coating seems to be better for and 316L-HA/gentamicin coating compare to 316L-HA coating for both bacterial strains.*

**Keywords**: drug release, antibacterial effect, hydroxyapatite, gentamicin, stainless steel

## 1.INTRODUCTION

Several decades after the discovery of the first antibiotic, bone infections still represent a major problem in medicine. Recent studies estimate the current incidence of infection to be around 2% and 4% in total hip and knee arthroplasties, respectively [1-7].

The figures can increase to 50%, due to pin track infections, when external fracture fixators are used in trauma surgery [8]. The reason lies in the poor accessibility of the bone-infected site by systemically administered antibiotics. Local therapy is therefore desired and can be achieved by using a suitable carrier for a controlled drug delivery. Calcium phosphates such as hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) are known to be biocompatible and osteoconductive, as they form an intimate bond with bone tissue after implantation, they might be suitable carriers for antibiotics. HA is the major mineral component of the bone, being also the cheapest solution for bone grafts, maxillofacial surgeries, dentistry bone defects repair in orthopedic interventions [9]. Bone are consisting of an organic phase - collagen up to 30 wt%, an inorganic phase almost 70 wt% and around 10 wt% of water [10]. The bare hydroxyapatite owns important properties, such as: low weight, tensile and fatigue strength [11], has no side effects on human organism [12] and bioactive properties: it has the capability to form strong chemical bonds with surrounding bone living

tissues [13], has osteogenic potential to promote bone growth, promote metallic implant integration with host bone. It can be obtained by different methods: from natural sources or via chemical synthesis [14].

However, organic molecules like antibiotics cannot be incorporated within the plasma-sprayed calcium phosphate coatings or HA ceramics, due to the extremely high processing temperature.

Kannan S. et al. [15] suggested that type 316L SS plays a key role in the bone replacement surgery due to its excellent mechanical features, availability at low cost and ease of fabrication. However it fails miserably in vivo conditions due to corrosion-related problems. Hence are alternative method on the development of hydroxyapatite (HA) coatings has been elucidated to impart corrosion resistance of the base metal and ensure biocompatibility of the ceramic on the metal surface. This also could not match the implant at the host site due to the continuous interaction of hostile environment with the implant and results in the dissolution of both ceramic and metal. An artificially induced passive layer on the metal surface prior to coating may improve the nature of implant on the resistance to corrosion. In the present study, the effect of $HNO_3$ treatments on 316L SS and the coatings on passivated 316L SS is being explored. Electrochemical studies involving cyclic anodic polarization experiments and impedance analysis in Ringer's solution were done to determine the corrosion resistance of the coatings.

Coating bone substitute materials with antibiotics should prevent adherence and colonization of bacteria at the biomaterial surface [16]; it might be a promising way to avoid postoperative infections. Local antibiotic prophylaxis by loading of filling biomaterials is a more relevant approach than providing systemic antibiotics.

Antibiotic coatings should be biocompatible and fully resorbable; they should not interfere with the intrinsic osteoconductive properties of the bone filling material and should not alter their bone substitutive potential.

## 2.MATERIALS AND METHODS

### 2.1.Materials.Sample preparations

The 316 samples were ground using a rotating device under a stream of water using 320 and 500-grit SiC paper. The composition of 316L is presented in table 1.

**Table 1.** Composition ranges for 316L stainless steels.

| grade | C | Mn | Si | P | S | Cr | Mo | Ni | N |
|---|---|---|---|---|---|---|---|---|---|
| 316 | 0.03 | 2 | 0.75 | 0.045 | 0.03 | 16 | 3 | 10 | 0.1 |

Samples were ground in one direction until all imperfections were removed, then the samples were cleaned ultrasonically in a bath of 50% ethanol/50% ultra-pure water and afterwards thoroughly rinsed with ultra-pure water [17].

The as-prepared AISI 316 samples were further used as substrates for film preparation using an

potentiostat/galvanostat Voltalab 40 equipment.

**2.2. Electrodeposition of HA**

For electrodeposition, the used electrolyte was a mixture between $Ca(NO_3)_2 \cdot 4H_2O$ (0.042 M) + $NH_4H_2PO_4$ (0.025 M) and gelatin as a chelating agent. The chemicals were provided by Sigma Aldrich. The electrodeposition process was carried out using amperometric techniques with a potential of -1.3 V and 60 min, at $65^0$C. Gentamicin was loaded by immersing the 316 coated (HA) samples in solution of 40 mg/ml gentamicin sulfate (General Drug House, Thailand) and keeping under vacuum for 10 mins.

**2.3. Surface analysis**

The structure morphology of covered 316 stainles steel was observed by field emission scanning electron microscopy SEM Hitachi S-4160. To identify the functional groups of the coating a Fourier transform infrared spectroscopy (FTIR) in transmission-FTIR mode was used in the range of from 400 to 4000 $cm^{-1}$, using a Spectrum 100 Series Spectrometer from Perkin Elmer.

**2.4. *In Vitro* Gentamicin Released from coating**

The elution study was employed to determine the release characteristics of antibiotics. A phosphate buffer saline (0.1 M PBS, pH 7.4) was used as the dissolution medium [18]. The coated samples were incubated in 100 ml of PBS at 37°C for 24 h. The dissolution PBS was collected after shaking four various time duration to calculate the concentration of gentamicin using a UV/Vis spectrophotometer (Perkin Elmer –Lamda 950). For absorbance measurement the wavelength maxima (λmax) was observed at 202 nm.

**2.5. The antibacterial effect** was determined using an UV–Vis Jenway Spectrophotometer, for determination optical density at 600 nm corresponding to the blank, the sample in infected media ($T_0$ and $T_1$ at time 0 and 24 hours) and positive control ($C_0$ and $C_1$ at time 0 and at 24 hours), used in equation (1) [19]:

$$I\% = \frac{(C_1 - C_0) - (T_1 - T_0)}{(C_1 - C_0)} \times 100 \quad (1)$$

This equation permits the determination of the growth inhibition index $I_{\%}$ of a negative gram bacteria as *E. coli* (ATCC 25922). and a positive gram bacteria as *S. aureus* (ATCC 25923) respectively. *Escherichia coli* is one of the first causes of Gram-negative orthopedic implant infections. *S. aureus* which is responsible for osteomelitys was regarded long time as a non-motile organism, but recently [20] it has been shown that it can move across agar surfaces.

According to the procedure described in literature [21] the coating samples were rinsed with distilled water and sterilized at 180° for 2 h, before the antibacterial assay. The concentration was fixed at 0.5 McFarland units measured with a McFarland densitometer. The amount of infected medium added in each sample was 10 mL and took place before incubation at 37° C, for 24 h.

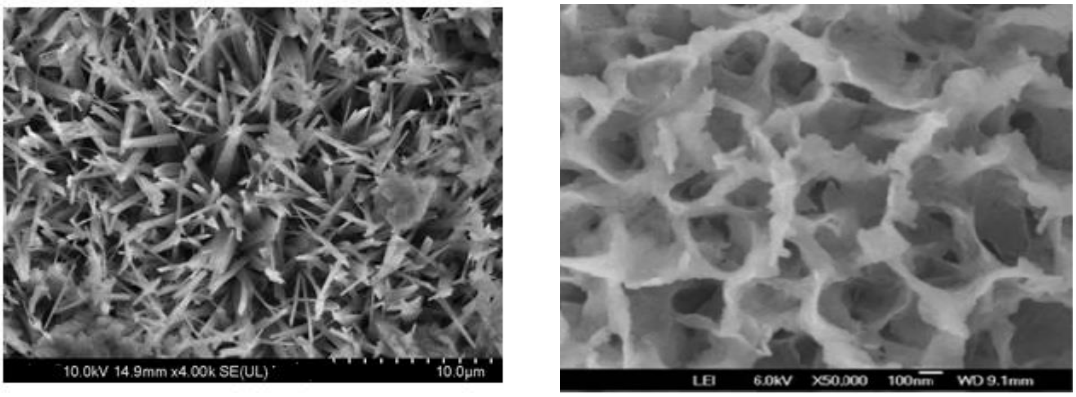
The blank was represented by saline solution and the positive control was represented by the infected medium without samples, and both of them were kept in the same conditions.

## 3.RESULTS AND DISCUSSION

### 3.1.Scanning electron microscopy

To study the morphology and size of coated HA with and without gentamicin on stainless steel substrate SEM was carried out. For The HA without gentamicin we can observe an acicular structure, like dandelion flowers on the surface of 316L SS specimen. By adding gentamicin, the surface morphology become different.

For the gentamicin sample, SEM observations denote a porous coating cauliflower-like, the sphere size goes from 15 to 25µm. (Fig. 1).
To measure the thickness of coating, cross-section of samples was studied under SEM. Figure 2 shows the SEM micrograph of the cross-section of coated 316 SS. It is seen from the micrograph that thickness is around 2 to 3 µm.




**a)** **b)**

**Fig 1.** Porous HA(a) and HA/gentamicin (b) coating on stainless steel specimen

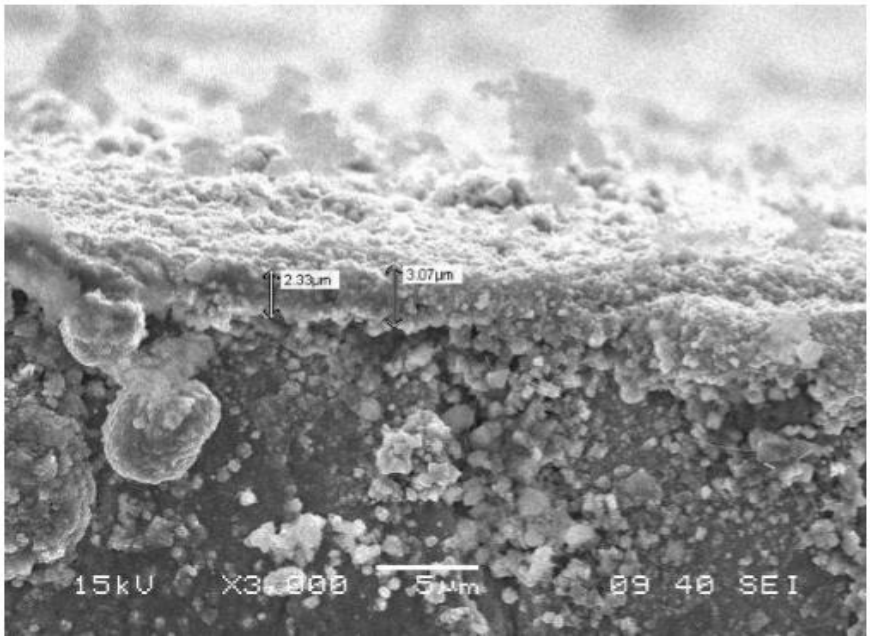



**Fig 2.** Cross-section on stainless steel specimen with HA/gentamicin coating

### 3.2.FTIR analyses

Fig. 3 shows the FT-IR spectra of HA coating with and without gentamicin. The characteristic bands of HA with the wide band near 3421 $cm^{-1}$ was ascribed to O–H stretching. The amide I adsorption of biopolymer at 1653 $cm^{-1}$ and amide II adsorption of biopolymer at 1550 $cm^{-1}$, the broad band adsorption of O– H in plane bending at 1382 $cm^{-1}$. The bands due to the PO stretching were absorbed strongly at 1033, 600 and 561 $cm^{-1}$ [22]. Generally, the characteristic bands of gelatin-HA composite were the combination of HA and gelatin. The characteristic adsorption band at 2937 $cm^{-1}$ was ascribed to the methylene group ($–CH_2$) symmetrical and asymmetrical stretching. Another characteristic adsorption band at 1120$cm^{-1}$ was ascribed to C–O stretching [23].

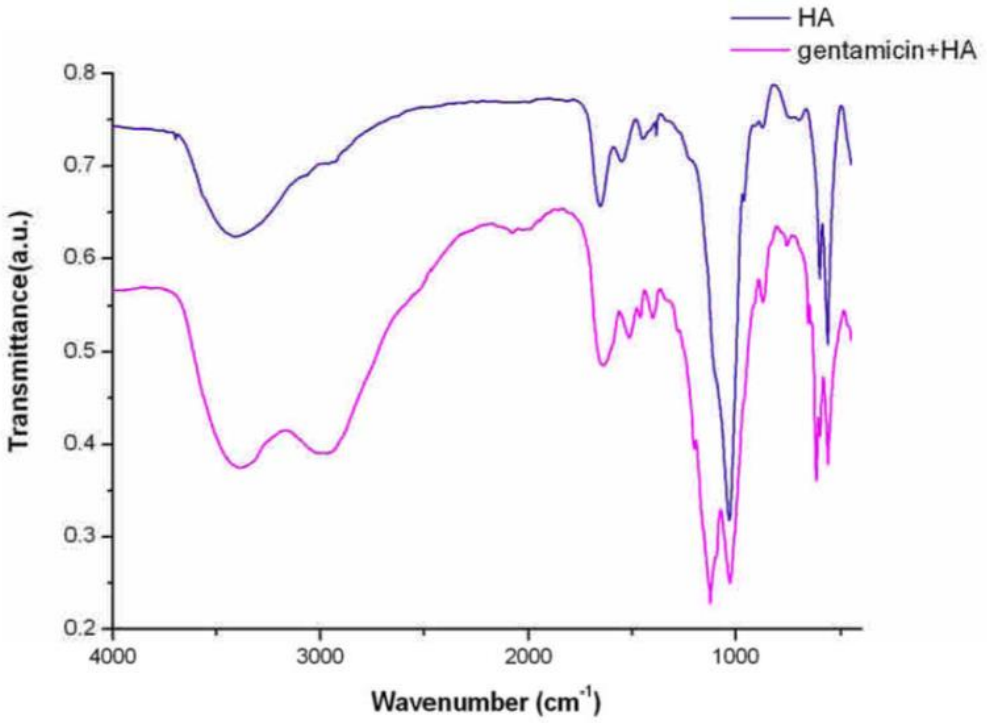



**Fig 3.** FTIR spectra on stainless steel specimen with and without HA/gentamicin coating

### 3.3.Drug Release

Kinetic release measurements were duplicated for coated samples. The released profile of gentamicin from the gentamicin-impregnated coating is presented in Fig.4. It was seen that the release of gentamicin followed a typical drug release profile, an initial burst release in a few days followed by a slow release over the next 3 weeks before reaching equilibrium [24]

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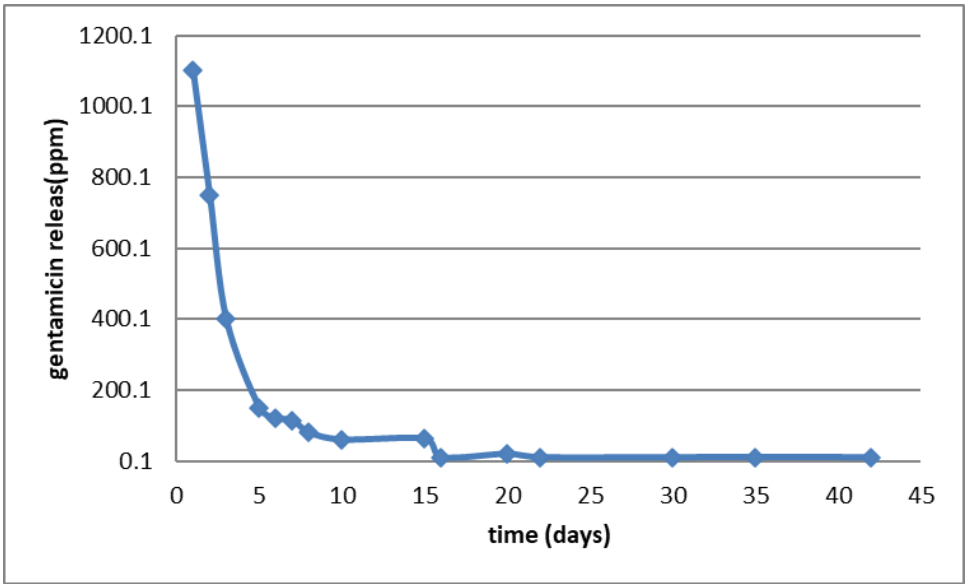


**Fig 4.** UV-Vis absorption curve for gentamicin from HA/gentamicin coating on 316L SS

### 3.4.Antibacterial effect

In table 2 the bacteria growth inhibition rate for the two samples is presented.The inhibition values which are close to each other are a bit higher for *S. aureus* compared to *E.Coli*.

For the coatings with gentamicin the inhibitions are higher.

**Table 2.** Antibacterial inhibition index

| I% | 316SS/HA | 316SS/HA-gentamicin |
|---|---|---|
| *E. Coli* | 37.45 | 68.25 |
| *S. aureus* | 42.84 | 77.15 |

Regarding antibacterial mechanism of action, it is to mention the general mechanism [25, 26] with two stages as following: an instantaneous and physical reversible phase, and a time-dependent irreversible molecular and cellular second phase. At the contact with a support as tissue, a competition between microbial colonization and tissue integration is taking place.

## 4.CONCLUSIONS

By adding gentamicin in a HA/316L SS sample, we obtain some benefits like a better morphology of the coatings, a better biocompatibility with implants, a better antimicrobial effect.

Biofilm embedded bacterial pathogens such as *S. aureus* and *E. Coli*, are major sources of bacterial infections and very difficult to be eradicated.

Investigation such as this paper could be useful for development future coatings able to prevent infections on stainless steel surface.

The best stability in bioliquids and best antibacterial effect recommend such coating for bio-applications in medicine.

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# MONITORING OF NITRATE CONCENTRATION IN DRINKING WATER- A CASE STUDY

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***Abstract***

*Water quality can be defined as a set of physical, chemical, biological and bacteriological characteristics. The theme of the paper is to monitor the concentration of nitrate ions in drinking water over a period of 3 months. The measurements were made daily for 3 months. For the days when no values were recorded, the data were simulated. Laboratory determinations were made according to standardized methods.*

**Key words :** drinking water, water quality indicators, nitrates

## INTRODUCTION

Water quality can be defined as a set of physical, chemical, biological and bacteriological characteristics, expressed in value, which allow a sample to fall into a certain category. To establish water quality, the analyzes of the most significant parameters are made [1-9], classified as follows: basic parameters: temperature, pH, conductivity, hardness, dissolved oxygen, dissolved salts, turbidity, analysis of cations and anions, colibacilli; indicators of persistent pollution: cadmium, mercury, organohalogen compounds and mineral oils; optional parameters: total organic carbon, biochemical oxygen consumption, anionic detergents, heavy metals, arsenic, boron, sodium, cyanides, oils and others [1-10].

The quality of drinking water is legislated by two laws: Law no. 458 of 08/07/2002 regarding the quality of drinking water, Law no. 311 of June 28, 2004 for the amendment and completion of Law no. 458/2002 on drinking water quality [1-9].

Drinking water quality parameters are microbiological, chemical and indicators. The maximum allowed concentrations according to the two laws of nitrates (chemical parameter) is 50mg / l, This nitrogen compound is the main sign of water pollution and exceeding the maximum allowed limit is a health risk factor [1-9].

The maximum allowed concentrations according to the two laws, of the nitrate ion concentration (chemical parameter), in drinking water is 50mg / l.

The theme of this paper is to monitor the concentration of nitrate ions in drinking water over a period of 3 months. The case analysis was based on the data obtained for the period October-December 2013 by the water company of Oradea. The measurements were made daily for 3 months. For the days when no values were recorded, they were simulated.

## EXPERIMENTAL

The principle of the method consists in the spectrometric measurement of the absorbance of the yellow compound formed by the reaction of sulfosalicylic acid (formed by the addition to the sample of sodium salicylate and sulfuric acid) with nitrate, followed by treatment with alkaline solution, [1-9].

The disodium salt of ethylenediaminetetraacetic acid (EDTANα$_2$) is added to the alkaline solution to prevent the precipitation of calcium and magnesium salts. Sodium azide is added to remove interference with nitrogen.

The reagents used are bidistilled water, $H_2SO_4$ and glacial acetic acid in the concentrations specified in the standard method, alkaline NaOH solution = 200g / l, disodium salt of ethylenediaminetetraacetic acid dihydrate (EDTANa$_2$) {[$CH_2$-N ($CH_2COOH$) CH2-$COONa_2$ 2H2O} 50g / l, control solution, $KNO_3$ standard has concentration, 1g / l. The control sample uses double distilled water. Read the absorbance of the samples compared to the control sample, of distilled water at λ = 415nm.

All reagents used are of purity for analysis.

## RESULTS AND DISSCUSSIONS

The monthly variation of the nitrate ion concentration is presented in figure 1. This representation allows highlighting some maximum points on the graph: how many times per month they appeared. In the first month it was just 1 day when the nitrate concentration overtake the recommended value for drinking water.

Figure 2 shows the values of nitrate ion concentration measured from water samples analyzed daily in November.

Figure 3 shows the values of nitrate ion concentration measured from water samples analyzed daily in December.

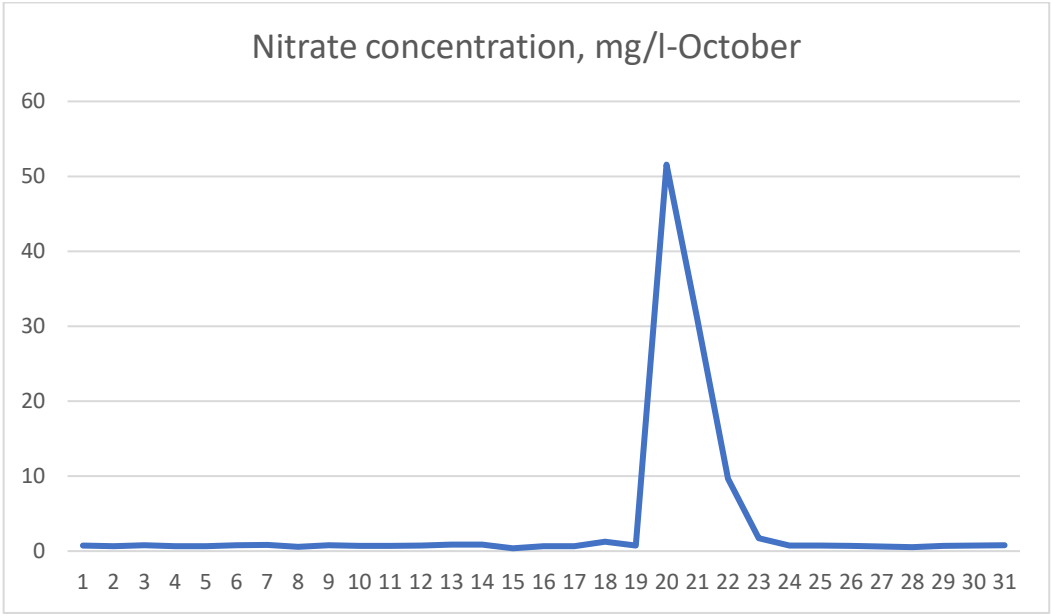



Figure 1. Daily monitoring of nitrate concentration in drinking water in october.

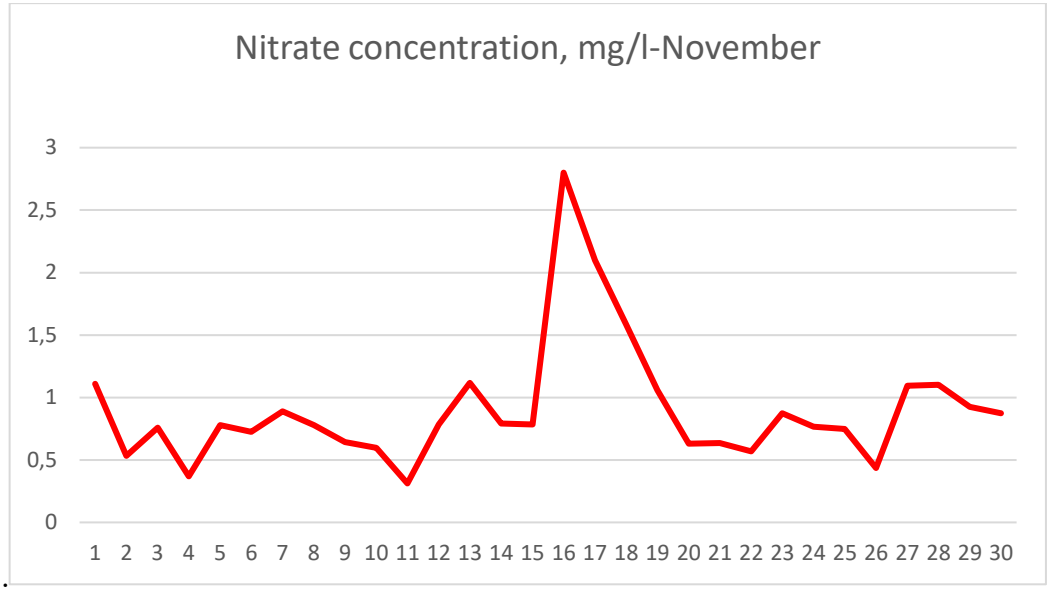



Figure 2. Daily monitoring of nitrate concentration in drinking water in november.

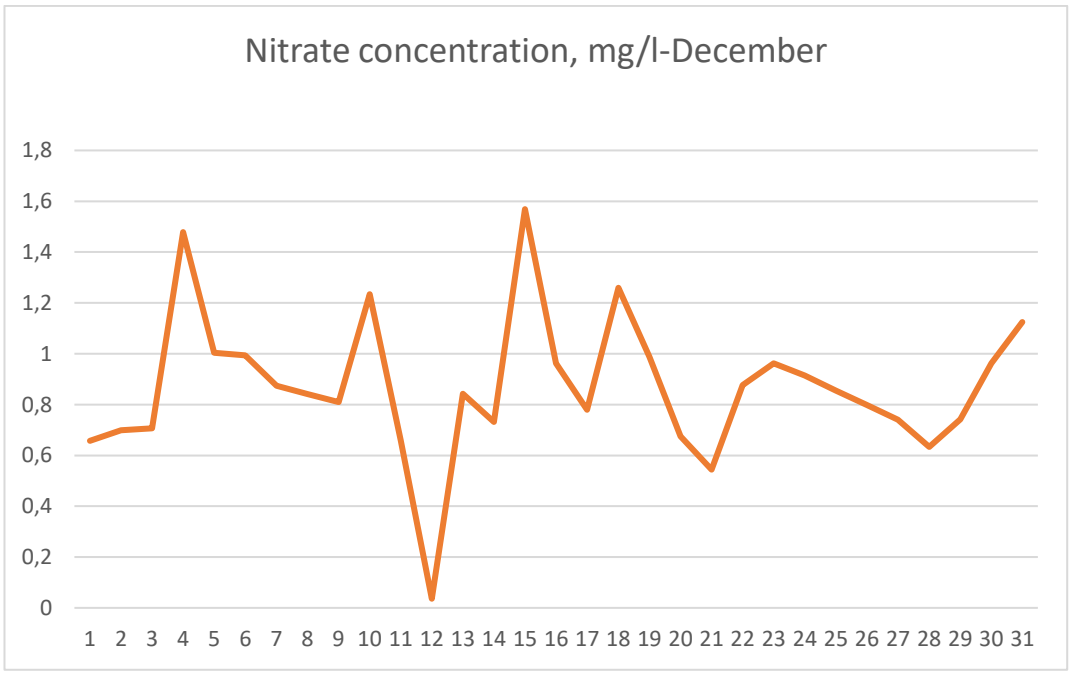



Figure 3. Daily monitoring of nitrate concentration in drinking water in december.

Table 1 and Figure 4 show the evolution of the monthly average value of the nitrate ion parameter in the three months analyzed, October, November and December. The values were calculated as an arithmetic mean and are 3.6622 mg / l in October, 0.9054 in November and 0.8694 mg / l in December, respectively.

All values are within the allowed limit.

Figure 5 also shows the share of confoem and non-compliant samples, for October - of 96.78% - respectively 3.22%, as well as for the entire studied interval, October-December, where the percentage of non-compliant samples is 1.08%.

Table 1. Monthly averages of nitrate ion concentration in drinking water

| Month | Nitrate concentration, mg/l-monthly average |
|---|---|
| October | 3,6622 |
| November | 0,9054 |
| December | 0,8694 |

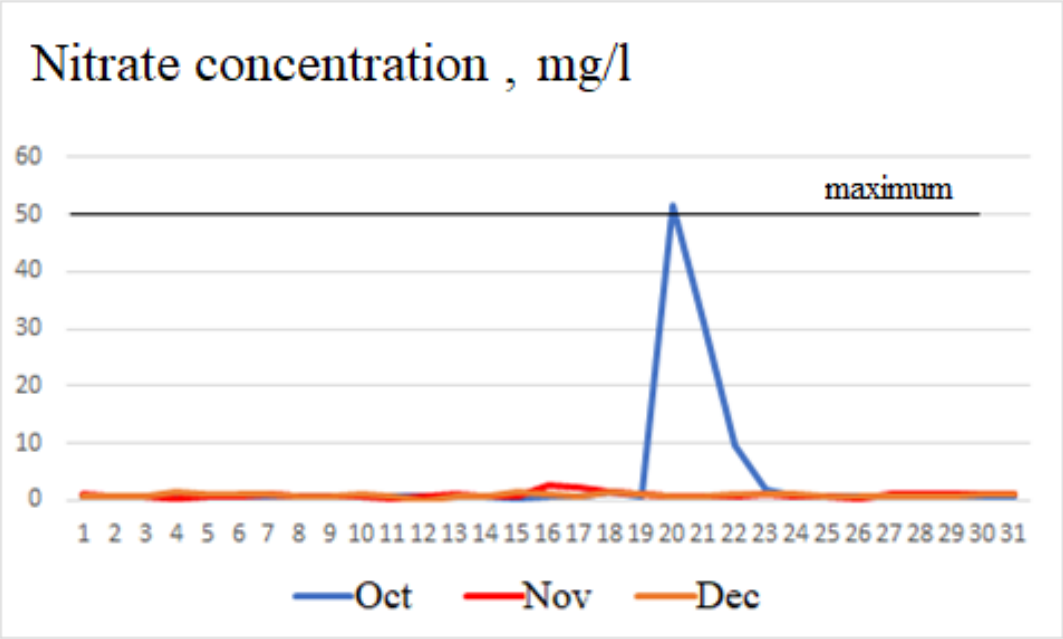
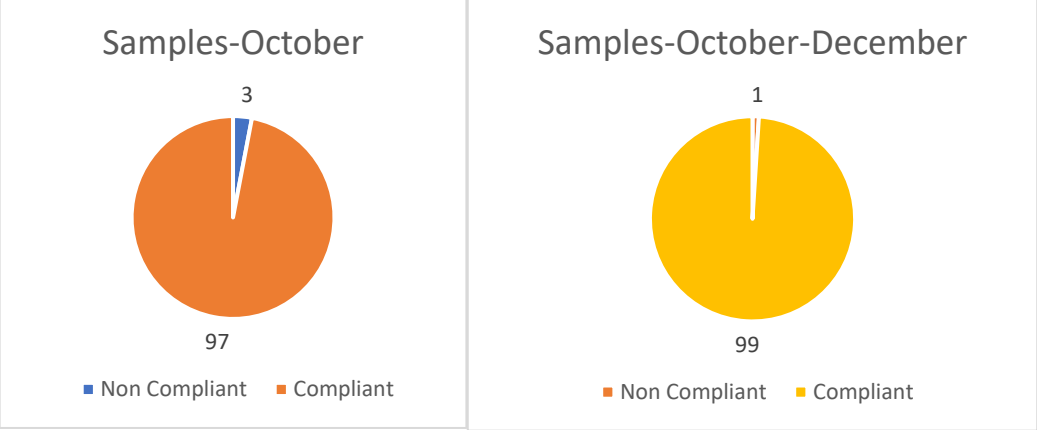



Figure 4. Comparative evolution of nitrate ion concentration in October-December, compared to the maximum allowed limit of 50 mg / l.




a) b)

Figure 5. Compliant vs non-compliant samples:
a) - October- 1 month, b) -October-December- 3 months

Figure 5 shows the monthly average values compared to the allowable limit of 50 mg nitrate ion / l. It is observed that the monthly average is well below the maximum allowed value, even if in the first month it was exceeded.

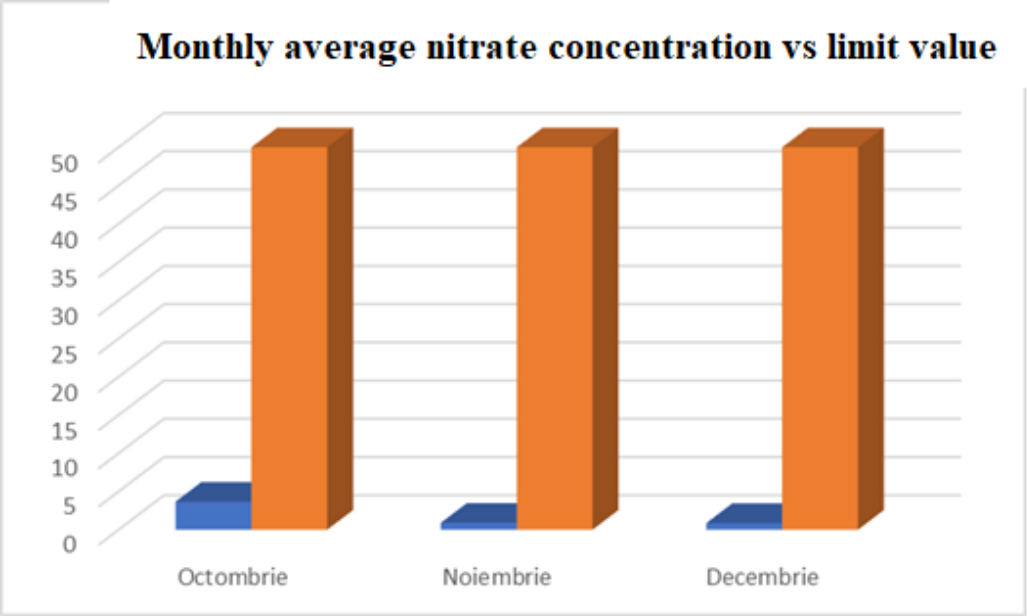



Figure 5. Monthly average nitrate ion concentration compared to the permissible limit value for drinking water

## CONCLUSIONS

From the comparative analysis of the values of the water quality parameter, namely "nitrate ion concentration" or "nitrates", determined in October-December, the following conclusions can be drawn:

- Nitrate values do not exceed the allowed limit of 50mg / l, with one exception in October, the 20th day, namely 51.569 mg / l
- It is observed that the monthly average of the nitrate ion concentration is well below the maximum allowed value, even if in the first month it was exceeded.
- The values were calculated as arithmetic mean of the daily determinations and are of 3.6622 mg / l in October, of 0.9054 in November, respectively of 0.8694 mg / l in December.
- The share of compliant samples is: for October of 96.78% - at 3.22%, and for the whole studied interval, October-December, 98.92 at 1, 08%.

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# SPECTROPHOTOMETRIC DETERMINATION OF Fe II IN AGRICULTURE SOIL FOR TOMATOES CROP

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***Abstract:*** *In the present study the aim was to determine the Fe II content in soil samples taken from the greenhouse of a tomato crop (from Oradea Town -NW Romania). The method used was a spectrophotometric determination in the visible range using 1.10 phenanthroline as a color reagent at a wavelength of 510 nm. The soil under study proved to be a sandy soil, with a rather low content of Iron II (0.7941 mg of $Fe^{2+}$ / 1 kg of soil) with a slightly acidic pH, soil which from these points of view is suitable for tomatoes cultivation.*
***Key words:*** *Iron II, spectrophotometric method, soil, o-phenanthroline*

## INTRODUCTION

One of the functions of the soil is to produce Phyto mass, which is used as a basic material for the production of food, clothing, fuel, etc. This function is due to the property of the soil to be a continuous reservoir and supplier of water and nutrients, which gives it fertility general property's. For plant growth, the soil provides many chemical elements necessary for the vegetation development and crop formation. 14 Of these, are considered nutrients. Depending on the amount needed by plants and their physiological and biochemical functions, nutrients are divided into macronutrients and micronutrients. Soils contain different nature reserves of nutrients depending on the nature of the parent material and the type of soil. Iron, like Aluminum, is a metal widespread in the earth's crust (its concentration being between 4.6% and 4.4% depending on the type of rock). In the soil the Iron concentration is between 0.2% and 55% (20,000 and 550,000 mg / kg) varying significantly from area to area [1].

It has generally been observed that sandy soils contain the lowest amounts of Iron while clay soils have the highest content of Iron. In the soil Iron can be present as Fe II or Fe III. The presence of iron in the soil in one state of oxidation or another is determined by certain factors such as pH or the presence of oxidizing conditions [2].

Iron is an essential element for plant development being considered a micronutrient because it is involved in biochemical processes absolutely necessary for plant cells. Therefore plants absolutely need to assimilate Iron from the soil. The divalent form is more soluble and therefore more available to be assimilated by plants. The iron requirement of plants depends very much from one species to another [3].

Oxidation processes of divalent iron to trivalent iron can take place in the soil under certain conditions (pH, the presence of oxidants) forming hardly soluble oxides or hydroxides, thus becoming unavailable to plants. In general, alkaline and oxidizing soils favor the formation of trivalent iron, while acidic and reducing soils favor the presence of divalent iron. Important

factors in the case of iron transformations in the two oxidation states are moisture and soil aeration [3].

Thus, in the case of agricultural land, it is very useful to monitor the iron content in order to ensure the optimal conditions for plant cultivation (by adding mineral fertilizers with iron content).

Cultivated plants need an optimal content of Iron in the soil in order to grow. An too low iron content can lead to underdevelopment of the plant (or even chlorosis) while an too high iron content can be toxic to the plant. The presence of iron ions in the soil above the allowable amount limit is caused by: the storage of solid and liquid waste on (under the soil) or discharges of effluents containing iron [4].

In the present work we studied the Fe II content in soil samples taken from an greenhouse of tomato (from a farm in Oradea town from NW of Romania).

## EXPERIMENTAL

### Spectrophotometric determination of Iron II in soil samples

### Principle of the method

The complex combination of Fe II with 1.10 phenanthroline, colored in red has a maximum light absorption in the visible range at 510 nm [5].

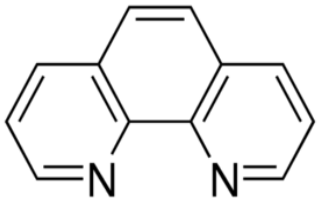


**Figure 1.** 1.10 phenanthroline https://pt.wikipedia.org/wiki/Fenantrolina

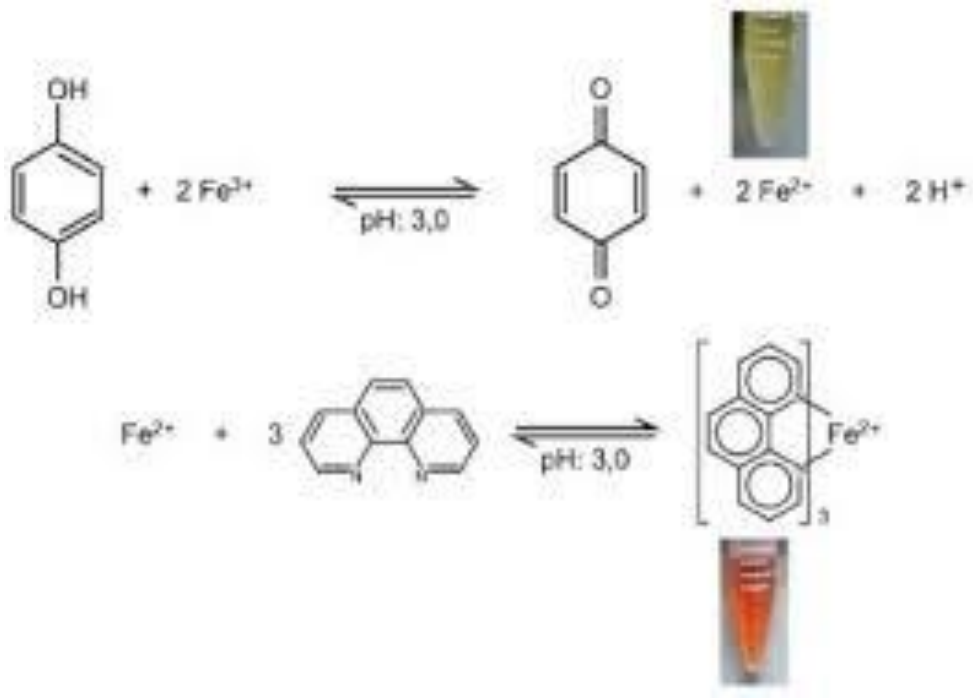


**Figure 2.** Fe II reaction with 1.10 phenanthroline quimicanova.sbq.org.br/detalhe_artigo.asp?id=6426

**Soil sampling**

5 soil samples were taken from a greenhouse with an area of approximately 500 $m^2$. Samples were taken from the center and the four corners of the study's stunning surface.

Soil samples were taken from a depth of 0-20 cm using a probe device. European standards were observed when sampling the soil [6].

**Plotting the calibration curve**

A series of standard Fe II solutions is prepared, at which the absorptions are measured, respecting the experimental conditions (preparation procedure, 1 cm tank thickness, 510 nm wavelength).

The measurement data are presented in the table below (standard series table).

The calibration curve correspond to: $A_{510\ nm}$= f(c).

**Sample processing**

Small amounts of soil samples are dissolved in water to determine soil pH. The pH of the analyzed soil was about 6. When processing soil samples, European standards were observed [7].

Over a quantity of 20 g of soil 50 ml of 5% $CH_3COONH_4$ solution were added to extract Fe II. The pH was adjust to pH = 4.8 by adding 2% nitric acid. The extract were stirred well for 30 minutes and then were filtered.

10 mL is taken from the extract, and treated with a 1% solution of hydroxyl amine hydrochloride to reduce Fe III to Fe II and then a 1,10-phenanthroline solution was added.

Absorbance was measured with a 1 cm cuvette at a wavelength of 510 nm for the 5 soil samples and the Fe II concentration determined by extrapolation from the calibration curve.

**Table 1.** Standard series

| C (mg/L) $Fe^{+2}$ | 0 | 2 | 4 | 6 | 8 |
|---|---|---|---|---|---|
| A | 0 | 0,160 | 0,313 | 0,460 | 0,619 |

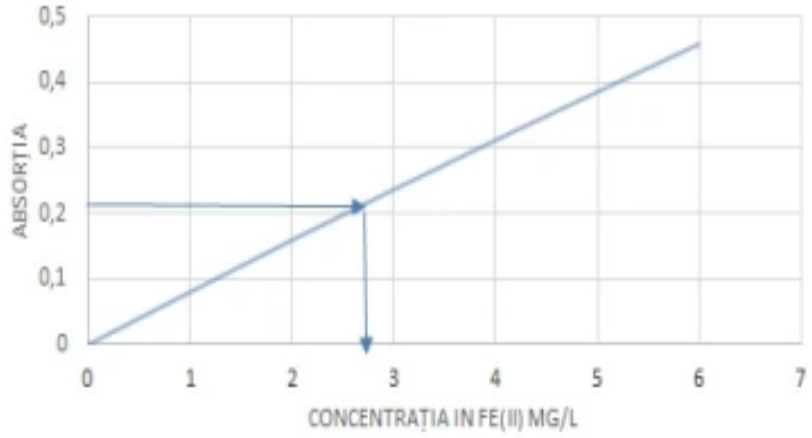


**Figure 3. Calibration curve**

**Table 2.** Fe II determination from soil samples result.

| **Soil sample** | **Absorbance** | **Fe II concentration** mg Fe (II)/L |
|---|---|---|
| 1 | 0,210 | 2,701 |
| 2 | 0,205 | 2,630 |
| 3 | 0,207 | 2,661 |
| 4 | 0,209 | 2,688 |
| 5 | 0,208 | 2,684 |

## CALCULUS.ASSUMPTIONS

*The soil moisture determined using the gravimetric method (oven drying at 110 ° C and repeated weighing to constant mass) is 15%.*

*Fe II concentration is considered to be 2.7 mg Fe II / L solution (approximately).*

The amount of Fe II (mg/kg dry soil) in the sample is calculated as follows:

**1. Calculus of the Fe II content in the 10 mL analyzed extract, if the concentration is 0,27 mg / L:**

1000 mL……0,27 mg de Fe II
10 mL……...x mg
x= 10mL 0,27 mg/1000
mL = 0,0027 mg de Fe II

**2. Calculus of the Fe II content in the 50 mL volumetric flask, where is the extract** obtained from 20 g of soil analyzed.

10 mL extract….0,0027 mg de Fe II
50mLextract……..y (mg)
y = 50mL. 0,0027 mg/ 10 mL = 0,0135 mg de Fe II
It's 5 times more!

**3. Calculus of the amount of dry soil from 20 g of soil, if the humidity is 15%:**

% dry soil =100%-% humidity
%dry soil=100%-15%
humidity=85%
m dry soil = 20 g .85/100=17 g

**4. Express the amount of Fe II in 17 g of dry soil as mg Fe II / kg soil:**

17 g dry soil…0,0135 mg de Fe II
1000 g dry soil……..z (mg)
Z = 1000 .0,0135/17 = 0,7941 mg of Fe II / kg of soil

## RESULTS AND DISCUSSIONS

Following the determinations performed for the evaluation of the Fe II content in the studied soil was obtained: 0.7941 mg of Fe II / kg of soil.

According to the data in the literature, in the investigated soil, the concentration of Iron II (0.7941 mg of Fe II / 1 kg of soil) is low (between 0.2% to 55% respectively 20,000 to 550,000 mg / kg- varying significantly from area to area [8]).

Thus we can conclude that the soil under study is a rather sandy soil, with a low Fe II content and a very slightly acidic pH (about 6).

## CONCLUSIONS

Tomatoes prefer medium soils, sandy-loamy, loamy-sandy, rich in humus, fertile, well-structured with good, deep drainage, with deep groundwater and a pH level between 5.5 and 7.0. Tomatoes grow and bear fruit well on soils rich in fertilizers: N total 0.12%, mobile $P_2O_5$ 1.5-20 mg per 100 g soil, exchangeable $K_2O$: 12-15 mg per 100 g soil, to which are added 5-6% nutrients from humus.

Tomatoes have high requirements for N, K medium for Mg and low for P and trace elements such as Fe II [9].

In conclusion, the soil under study is a sandy soil, with a rather low content of divalent iron and a slightly acidic pH, a soil that from these points of view is suitable for growing tomatoes.

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# INFLUENCE OF MANGANESE IONS OF CHLOROPHYL CONCENTRATION FROM WHEAT GRASS

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## Abstract

*In this paper we aimed to observe the extent to which manganese cations influence the formation of chlorophyll in the leaves of wheat seedlings. Moreover, we wanted to see how important it is if manganese ions are free or complex and if there are differences between the results obtained. The influence of $MnCl_2$ , $K_7PMo_{11}O_{39}$ and $K_5MnPMo_{11}O_{39}$ solutions was followed of 0,1 $\mu M$, 1 $\mu M$, 10 $\mu M$ on the concentration of chlorophyll and carotene in the leaves of wheat seedlings in the first days of life.The study shows that the formation of chlorophyll is a complex process to which obviously contributes to some extent manganese ions, but also other trace elements such as potassium, phosphorus and molybdenum.*

**Keywords***:* chlorophyll, manganoenzymes, manganoproteins, carotene, polyoxometalates

## INTRODUCTION

Aristotle argued that plants absorb organic matter through the roots of the earth, and this idea was maintained unchanged until the Middle Ages. In 1620, the Dutch researcher Van Helmont (1620) cultivated a cutting in a certain amount of soil. After a few years he compared the mass of the plant and the soil and found that the soil mass remained unchanged, so he deduced that the plants feed on the water with which they were sprayed. Another researcher, Joseph Priestley (1771) observed the change in the air content under a glass bell, in the presence of an animal, a plant or both. He found that plants "restore" the vitiated air by animals. Experiments with other plants such as Melissa officinalis, Spinacaia oleracea have led to the same result. [1,2]

Photosynthesis is the process by which plants synthesize from mineral substances, organic substances using light energy. The term was introduced by Pfeffer and came from the Greek words "fotos" = light and "synthesis" = combination, synthesis. Plants that produce their organic substances are called photoautotrophs. Other autotrophic plants are called chemoautotrophs, they produce their organic substance from mineral substances, using the energy from the oxidation of mineral substances. [3]

Photosynthesis to be explained as a transformation of light energy into chemical energy embedded in plant constituents. In green plants the main reactants are $CO_2$ - the ultimate electron acceptor and $H_2O$ - the electron donor. [3,4]

Manganese is an essential microelement for plant and animal life, it is found in the earth's crust in the form of insoluble oxides. Its importance in living systems is considerable, its the most important role is the active center of photosystem II, where the oxidation of water to oxygen occurs. Manganese is very widely involved in many biological functions, being a component of manganese enzymes and manganese proteins.

The most important role of manganese in living systems is the participation as an active center in photosystem II in which the oxidation

of water to dioxide takes place, according to the reaction:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

This process takes place on a manganese protein, which has four manganese atoms, the structure and location of the four manganese atoms has not been completely elucidated crystallographically. By spectroscopic methods the above reaction steps were established as monoelectronic oxidation steps. [5]

In some plant tissues for example, thorns from the common nettle there are large deposits of manganese in the form of $Mn_2P_2O_7$ (manganese diphosphate) along with deposits of $Ca(C_2O_4)$ (calcium oxalate) . Bacteria that store manganese in the form of $MnO_2$ are known, for example Gallionella, Sphacrotilius, Leptothrix and Chonothrix. Bacteria are thought to take up soluble manganese (II) salts, which are stored by precipitation and hydrolysis at a biological pH. [6]

Manganese is the most abundant element found in soils developed from iron-rich rocks due to its association with this element. It is found in the soil in the form of $Mn^{2+}$ or $Mn^{3+}$ ions . Organic chelates derived from microbial activity, degradation of soil organic matter, plant residues, and root exudates can form metal complexes with micronutrient cations, and thus increase the solubility and mobility of Mn cations. The availability of Mn to be adsorbed by plants is influenced by soil pH, which decreases as the pH increases. Divalent manganese is the form of manganese absorbed on the surface of the cell membrane in the root of the plant. If the pH of the soil decreases, the transfer of $Mn^{2+}$ increases greatly, while the oxides of Mn, Mn bound to iron decrease. Mn-oxidizing germs are the most effective oxidizing biological $Mn^{2+}$ systems in neutral and slightly alkaline soils. Relatively, with increasing soil pH, chemical immobilization of $Mn^{2+}$ increases, and chemical self-oxidation predominates at pH greater than 8.5 - 9.0. [6]

It has been observed that manganese stimulates plant growth and that it acts as a catalyst in plants, it is essential for plant life. [7]

Manganese is involved in many biochemical functions, primarily acting as an enzymatic activator for dehydrogenases, transferases, hydroxylases and decarboxylases involved in respiration, synthesis of amino acids and lignin, and regulation of hormone concentration, but in some cases can be replaced by other metal ions (eg Mg). Manganese is also involved in redox reactions in the electron transport system in plant photosynthesis. Manganese is also involved in the photosynthetic evolution of $O_2$ in chloroplasts for example the Hill reaction. Given the key role in this essential process, inhibition of photosynthesis occurs even at a moderate manganese deficiency; however, it does not affect the ultrastructure of the chloroplast or cause the chloroplast to rupture until a severe deficiency is reached. [6,7]

In this paper we aimed to follow how the free manganese ion or complexed with a phosphomolybdate type polioxoanionic ligand, respectively the free lacunar phosphomolybdate ion influences the concentration of chlorophyll in wheat leaf in the sixth day after the seeds were planted it germinated in the semi-darkness and on the eighth day of its life, after the wheat seedlings had been brought to light for two days.

## MATERIALS AND WORKING METHOD

The study was conducted comparatively for ten wheat seed germinators. For one of the germinators, distilled water was used as a wetting solution and was used as a control sample. For each group of three germinators, solutions of $MnCl_2 \cdot 4H_2O$, $K_7PMo_{11}O_{39} \cdot 17H_2O$ and $K_5[MnPMo_{10}O_{39}(H_2O)] \cdot 12H_2O$ of concentrations were used, in different concentrations from 0,1 μM, 1,0 μM and 10 μM.

The wheat seeds used were rigorously selected from a high quality assortment with germination capacity over 94%, 15% humidity, since last year production. The seeds were ventilated, washed for three times with distilled water, lightly tamponed with a napkin to remove excess of water and soaked for one hour at room temperature on a filter paper. dried at room temperature.

200 ml 0.01M $MnCl_2$ solution was prepared by dissolving 0.3958 g $MnCl_2$ in a 200 ml volumetric flask. 0.25 ml of this solution were removed to prepare 250 ml with distilled water to prepare the 10 μM solution. Through repeted dillution processes with respect to a volumetric rate 1:10 were prepared the next 1 μM and 0,1 μM 1 μM and 0.1 μM $MnCl_2$ solutions.

It was dissolved in a 100 ml volumetric flask *0.2322 g* $K_7PMo_{11}O_{39} \cdot 17H_2O$ necessary to prepare a 1 mM solution. Taking 2 ml of this sample and dissolving in a 200 ml volumetric flask in distilled water 10 μM $K_7PMo_{11}O_{39} \cdot 17H_2O$ solution required for the experimental part was prepared. The 1 μM and 0.1 μM solutions of $K_7PMo_{11}O_{39} \cdot 17H_2O$ were prepared in the same way as those of $MnCl_2$ .

For preparation of 1mM phosphomolybdate manganese complex solutions 0.2100 g $K_5[MnPMo_{10}O_{39}(H_2O)] \cdot 12H_2O$ was disolved in 100 ml distilled water. 10 μM $K_5[MnPMo_{10}O_{39}(H_2O)] \cdot 12H_2O$ solution was obtained after dillution of the previous solution. For both 1 μM and 0.1 μM $K_5[MnPMo_{10}O_{39}(H_2O)] \cdot 12H_2O$ solutions the same procedure using for the other two compounds should be followed, diluting 1:10 with distilled water each sample.

Ten germinators lined with filter paper were prepared. In the first germinator, the filter paper is moistened with 25 ml of distilled water. The wheat grains were placed neatly in germinator. Each germinator is sealed, so as not to lose moisture and stored in a semi-dark, insulated room at a constant temperature, at 21-23 °C. The samples were left to germinate for 4 days. On the fourth day the germinator were opened and the germinated grains are counted. On the sixth day, the germinators were opened, the seedlings were counted again, and samples of green leaves were collected to be used further at chlorophyll determination. The rest of the seedlings are moistened again with distilled water and left for another two days in the light under the conditions mentioned above. On the eighth day, the germinators are opened and leaves are harvested again to determine chlorophyll concnetration. [8,9]

Proceeded in the same way for samples treated with 0,1 μM, 1.0 μM and 10 μM of $MnCl_2 \cdot 4H_2O$, $K_7PMo_{11}O_{39} \cdot 17H_2O$ and $K_5[MnPMo_{10}O_{39}(H_2O)] \cdot 12H_2O$.

***Determination of chlorophyll concentration.*** The determination of the concentration of chlorophyll in the leaves can be

carried out in the following two ways: the chromatographic method or the photocolorimetric method. In the photocolorimetric method, the chlorophyll from the green leaves is solvated in suitable solvents and the absorbance for different wavelengths is determined. The values obtained for absorbances at the three wavelengths will be introduced in mathematical relations also called Lichtenthaler's formulas. Both the wavelengths and the constants in Lichtenthaler's formulas differ from one solvent to another [10].

An impediment of the photocolorimetric method is the very small difference between the absorption maxima of chlorophyll A and chlorophyll B. The maximum absorbance of pure chlorophyll A in acetone is 662.6 nm and the maximum absorbance for pure chlorophyll B is 645.6 nm. Because the two absorption maxima of chlorophyll A and chlorophyll B are very closed, there is an overlap of the two signals, so that at 662.6 nm and chlorophyll B will have a significant absorbance, and at 645.6 nm will absorb and chlorophyll B. The calculation formulas proposed by Lichtenthaler solve this problem.

Another problem that arises in the methods of determining chlorophyll is to remove the interference of degradation products. This is done by acidification of chlorophyll extracts. Upon acidification of chlorophyll, magnesium ions are removed from the porphyrin ring and pheophytin is produced. Acid degradation of chlorophyll occurs completely in 90 seconds and the maximum absorption of chlorophyll A can be reduced by half, while that of chlorophyll B is reduced by 15%. Chlorophyll A is the major pigment, and chlorophyll B in higher plants has a lower concentration, in many of them it is around a quarter of the chlorophyll A concentration. Other pigments found in green leaves are called carotenoids, but their color is masked by the intense green color of chlorophyll. The colors of the leaves pigments are:

- Chlorophyll A is greenish-yellow
- Chlorophyll B is bluish green
- Carotene is orange
- Xanthophyll is yellow.

***Procedure.***Samples of cutting leaves with a scissors from wheat seedles were weighted on a analytical balance. The weight was aproximatly 0.02 g for each sample. The chopped leaf is placed in a mortar with a pistil. A pinch of solid calcium carbonate spatula was added and with a pistil the mixture was crushed, compacted and homogenized until it becomes smooth. To the mixture of mortar was gradually added by pipetting 10 ml of acetone. Mix the solvent with the solid sample until the entire amount of chlorophyll is extracted from the solid material. The chlorophyll extract thus prepared was filtered, and the clear filtrate was collected in a container which was subsequently closed to avoid concentration of the extract by evaporation of the solvent. From the resulting filtrate, 2 ml were placed in two vats in a row. 0.01 ml of 0.5 N HCl was added to the second cuvette. The disappearance of the green color was observed and the solution changed to a yellow coloration specific to xanthophyll.

The T60 spectrophotometer reads the absorbances of chlorophyll solutions against acetone at 470, 645 and 662 nm. The spectra of chlorophyll extract solutions in acetone are shown in Figure1 . In both spectra you can see the disappearance of absorption maxima following acid degradation with hydrochloric acid.

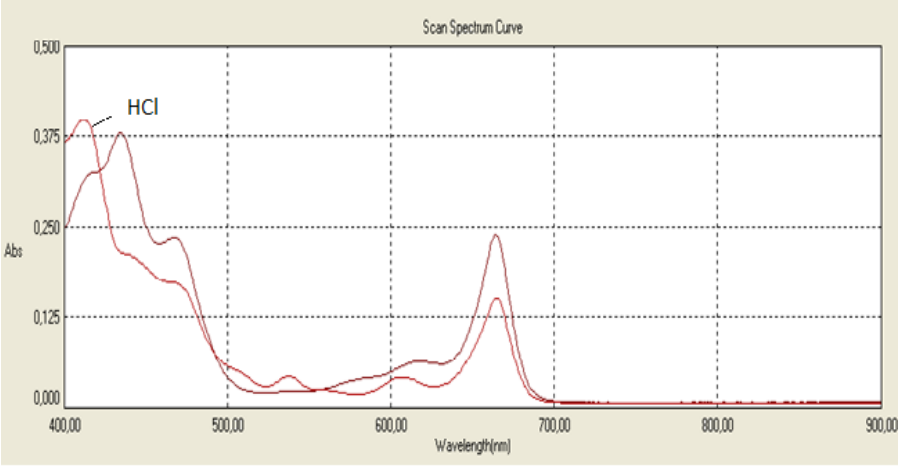


*Fig. 1. Absorption spectra of chlorophyll extract from wheat leaves in acetone*

## RESULTS AND DISCUSSIONS

The determination of chlorophyll A, chlorophyll B and carotene concentrations was performed using the calculation formulas proposed by Lichtenthaler. The concentrations of chlorophyll A and chlorophyll B in the prepared extract are determined by formulas (1) and (2), in which the concentration is expressed in mg of chlorophyll in one liter of solution. The carotene concentration obtained by means of formula (3) was also expressed in mg carotenoids per liter of prepared solution. We decided to express the concentration of chlorophyll and carotenoids in the leaves taking into account the mass of the sample under study, so we calculated concentration of chlorophyll and carotenoids mg to 100 g of leaves from formulas (4- 6) . We expressed total chlorophyll as the sum of the chlorophyll A and B concentrations resulting from the Equation 7. [11,12]

$$C_{extract}\ A\ (mg/L) = 11{,}75\ A^{662} - 2{,}35\ A^{645} \quad (1)$$

$$C_{extract}\ B\ (mg/L) = 18{,}61\ A^{645} - 3{,}96\ A^{662} \quad (2)$$

$$C_{extract}caroten(mg/L) = (1000\ A^{470} - 2{,}270\ C_{extract}A - 81{,}4\ C_{extract}B)/227 \quad (3)$$

$$Chl.A\ (mg\%) = \frac{C_{extract\ A}}{m_{sample}} \quad (4)$$

C extract B - is the concentration of chl $Chl.B(mg\%) = \frac{C_{extract\ B}}{m_{sample}}$ (5)

$$C_{carotene}(mg\%) = \frac{C_{extract\ caroten}}{m_{sample}} \quad (5)$$

$$\text{Total Chl.} = \text{Chl. A} + \text{Chl. B} \quad (6)$$

Chl. A - is the concentration of chlorophyll A (mg/100 g green leaves)

$C_{extract}$ A - is the concentration of chlorophyll A resulting from photocolorimetric determinations according to formula (1), in mg/L.

$m_{sample}$ - is the mass of leaf taken in working (g)

Chlorophyll B resulting from photocolorimetric determinations according to formula (2) expressed in mg / L in formula (5).

$C_{carotene}$extract - is the concentration of carotenoids resulting from photocolorimetric determinations and calculations, according to the formula expressed in mg/L.

The results obtained after performing the concentration calculations of chlorophyll A, chlorophyll B and carotenoids on the sixth day after preparing the samples and keeping them in the dark, for the extraction method with acetone for the leaves of the seedlings from the ten germinators are recorded in Fig.2.

In Fig. 2 it can be observed that in all wheat samples chlorophyll A is found in higher concentration than other components like chlorophyll B and carotene. The highest concentrations of chlorophyll A were recorded in samples treated with 10 μM manganese solution and 0.1 μM chelating ligand, $PMo_{11}O_{39}$ 0.1 μM. In manganese solutions taken in study, the increase of chlorophyll was favored at 1-10 μM concentrations, while in experiments driven in chelating ligand solutions, $PMo_{11}O_{39}$, the concentration of chlorophyll decreased with increasing of concentration from 0.1 to 1.0 μM. The use of a complex of the two studied components $MnPMo_{11}O_{39}(H_2O)$ slightly favored the increase of the concentration of chlorophyll A at concentrations of 0.1-1 μM. Chlorophyll B was higher than that obtained from the water, especially in the use of 0.1-1.0 μM monolacunar ligand $PMo_{11}O_{39}$ and its manganese complex 0, 1 μM $MnPMo_{11}O_{39}(H_2O)$ solution. In contrast, the carotene concentration was lower in all studies compared to the sample treated with distilled water.

At acid degradation (Fig. 3) high values of xanthophylls and carotene are still maintained when the samples were treated with 0,1-1,0 μM $PMo_{11}O_{39}$ and 1,0-10 μM $MnCl_2$ solutions. The decrease of xanthophyll concentrations is considerable when using $MnPMo_{11}O_{39}$ ($H_2$ O) complex solutions throughout the studied concentration range. The concentration of carotene them again maintained below that the water samples for all the solutions studied.

For a comparative study, the samples were kept for two more days in natural light, and then the concentration of chlorophyll and carotene in the leaves was determined as in semi-darkness, and the calculations of concentrations were performed with mathematical formulas ( 1-7 ) .

On the eighth day, it can be seen in Fig. 4 that chlorophyll A in all samples recorded more positive values than the control sample, the highest concentrations were obtained in 0,1 μM $PMo_{11}O_{39}$ and 1 μM $MnPMo_{11}O_{39}(H_2O)$. Spectacular increases of chlorophyll A concentrations were recorded in both studied polyoxometalates compounds. With regard to the concentration of chlorophyll B, it did not show such a large evolution compared to the control sample, the highest value was recorded when using 0,1 μM $PMo_{11}O_{39}$ solution. The carotene concentration does not appear to have been affected by the use of polyxometalates and manganese solutions compared to the control sample.

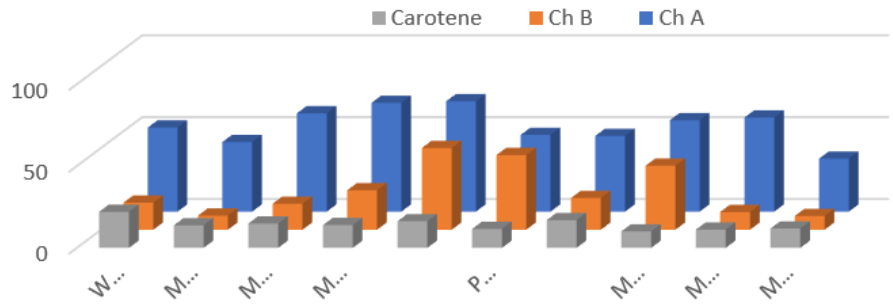


*Fig. 2 . Variation in concentrations of chlorophyll A, chlorophyll B and carotene in the leaves on the sixth day after seedlings are kept in semi-darkness*

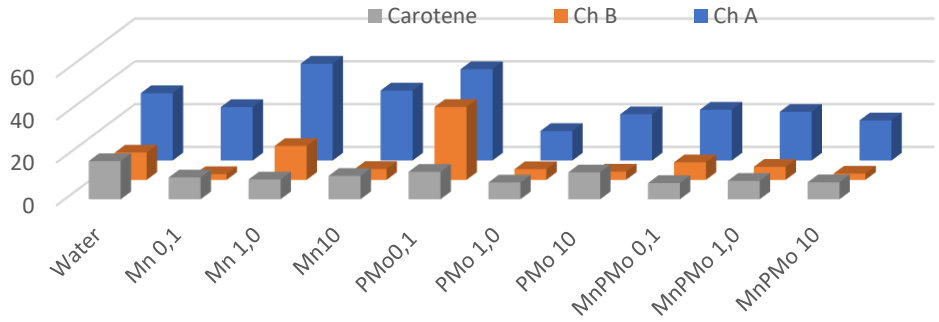


*Fig. 3 . Variation in the concentrations of* xanthophyll *A,* xanthophyll *B and carotene in the leaves on the sixth day after keeping the seedlings in the dark and treating the solutions with HCl*

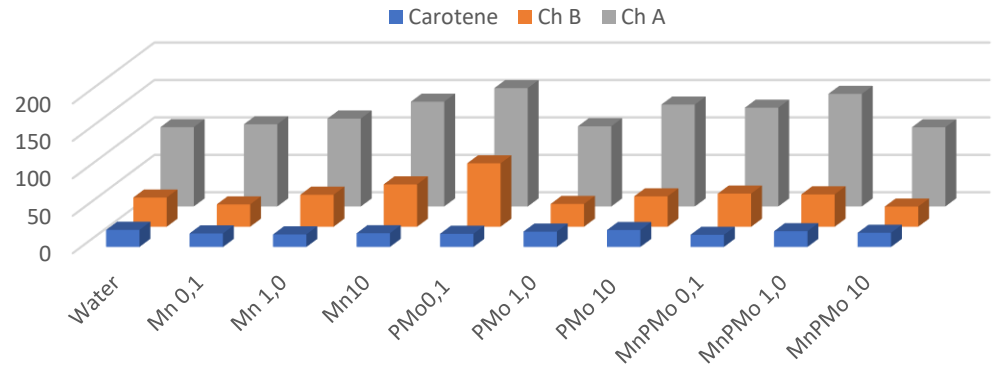


*Fig. 4 . Variation in the concentrations of chlorophyll A, chlorophyll B and carotene in the leaves on the eighth day after seedlings are brought to light*

In Fig. 5 it can be seen that following the degradation of chlorophyll samples with HCl, the concentration of chlorophyll B becomes very low, falling below that of carotene, while the concentrations of chlorophyll A remain higher than that of the control sample, especially when using 0, 1-1.0 μM $MnPMo_{11}O_{39}(H_2O)$.

In Fig. 6 it can be seen that the passage of the samples to light led to a substantial increase in the concentration of chlorophyll A, even after degradation with HCl. 10 μM $MnCl_2$ and 0.1-1.0 μM

polyoxometalates solutions stimulated in a higher proportion the increase of the concentration of chlorophyll A than.

Instead, in Fig. 7 it can be seen that the variation of the chlorophyll B concentration from the sixth day to semi-darkness to the eighth day in the light had a much more heterogeneous evolution. Chlorophyll B concentrations increased at light for absolutely all samples, but the increase was very diverse from each solution to other. No significant variations were observed in the use of 1,0 µM $PMo_{11}O_{39}$ and 0.1 µM $MnPMo_{11}O_{39}(H_2O)$. However, the most important values of chlorophyll B in light were obtained on the eighth day for samples treated with 10 µM $MnCl_2$ and 0.1 µM $PMo_{11}O_{39}$ .

The carotene concentration increased gradually, but without large jumps between values from the 6th day to semi-darkness to the 8th day after the seedlings were kept in the light. In the case of carotene, the presence of the studied substances did not appear to stimulate a concentration increase of carotene in the seedles leaves. Moreover, in semi-darkness it can be said that the carotene production was even slightly inhibited especially in the presence of $MnPMo_{11}O_{39}(H_2O)$ complexe.

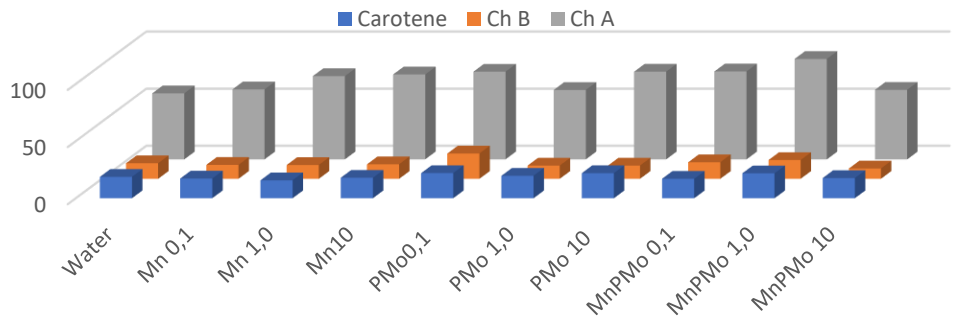


*Fig. 5 . Variation of xanthophyll and carotene concentrations in leaves on the eighth day after seedlings are brought to light and degradation with HCl*

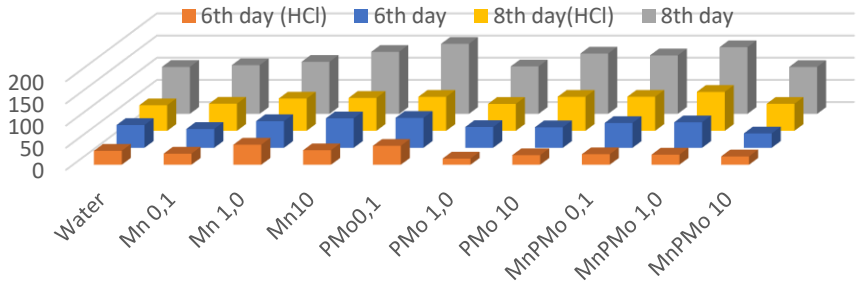


*Fig. 6 . Variation of chlorophyll A concentrations in wheat leaves from day six to semi-darkness to day eight to light*

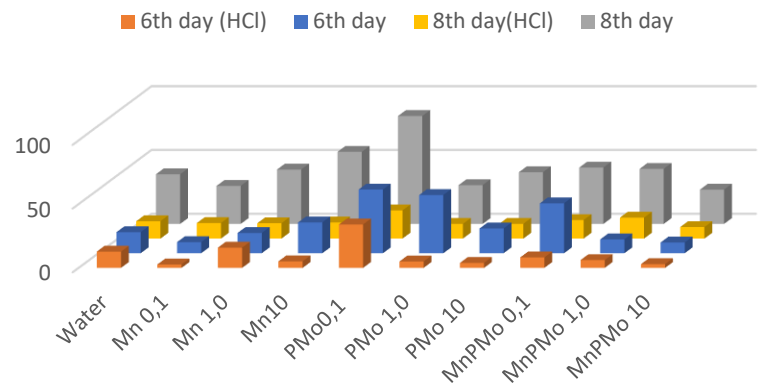


*Fig. 7 . Variation of chlorophyll B concentrations, in wheat leaves from day 6 to semi-darkness to day 8 to light*

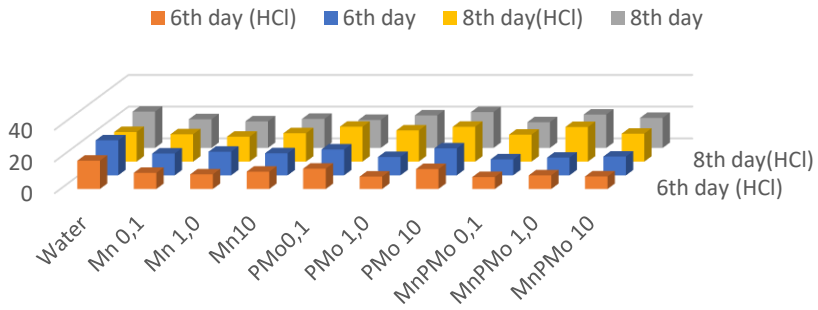


*Fig. 8 . Variation of carotene concentrations in wheat leaves from day 6 to semi-darkness to day 8 to light*

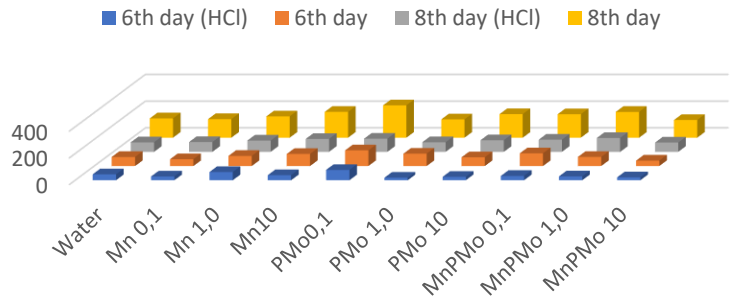


*Fig. 9 . Variation of total chlorophyll concentrations in wheat leaves from day six to semi-darkness to day eight to light*

The variation in total chlorophyll concentration was recorded in Fig. 8 . Total chlorophyll increased significantly on the 8th day after retention of light samples in absolutely all solutions studied. The use of monolacunar POM (polyoxometalate) by 1.0 μM concentration has proven to be most effeciantly in stimulation of the chlorophyll concentration increase. Instead, the 10 μM concentration of the both POMs led to an inhibition of the increase of chlorophyll concentration compared to the control solution.

## CONCLUSIONS

The aim of this paper was to study the influence of $MnCl_2$ , $K_7PMo_{11}O_{39}$ and $K_5MnPMo_{11}O_{39}$ by 0.1 μM, 1 μM, 10 μM through to the concentration of chlorophyll and carotene in the wheat seedlings leaves in the first days of life. Interpretation

of the results and discussions were performed compared to a control sample, in which only distilled water was used to sprinkle wheat seeds and seedlings. Based on the results obtained, the following conclusions were reached : in the sixth day after the preparation of the germinators, it was found that all the seeds had green leafy seedlings, which means that the concentration range chosen for the three solutions was beneficial, and there was no inhibition of growth and development. It has been observed that keeping the leaves in the light for two days, from the sixth to the eighth day leads to a significant increase, going to a doubling of chlorophyll A and B concentrations. Measurements in the presence of HCl show that the increase is significant especially in type A xanthophyll and to a lesser extent in type B.

The chlorophyll concentration was increased when using manganese solutions with concentrations higher than 1 μM. These solutions stimulated the formation of chlorophyll A and B much better in the presence of light. In the case of the use of the chelating ligand $K_7PMo_{11}O_{39}$ , only the 0.1 μM concentration solution stimulated the synthesis of chlorophyll and xanthophyll respectively in the first six days, instead in light, the chlorophyll concentration increased when using the chelate in the whole concentration range. studied. The largest increase in chlorophyll concentration was recorded for the $K_7$ $PMo_{11}$ $O_{39}$ 0,1 μM solution.

When using complex $K_5MnPMo_{11}$ $O_{39}$ solutions containing both manganese and the studied chelate, the stimulation of the increase of chlorophyll concentration after the semi-dark period were not as significant as when using $MnCl_2$ and $K_7PMo_{11}$ $O_{39}$ . In contrast, after two days of keeping the seedlings in the light, there is even an improvement. The reasons for the decrease in chlorophyll concentration when using the $K_5MnPMo_{11}$ $O_{39}$ complex could be the following: Higher stability of the $K_5MnPMo_{11}$ $O_{39}$ complex compared to the chelate $K_7PMo_{11}O_{39}$ , which means that it releases a lower concentration of component trace elements (phosphate from phosphate, molybdenum from molybdate); manganese cations are bound inside the complex, they are no longer free as in manganese chloride; the number of potassium cations has decreased; it is possible that on the eighth day, the seedlings being more developed and benefiting from the presence of light to streamline the way they extract nutrients from the solutions of the $K_5MnPMo_{11}$ $O_{39}$ complex.

The study shows that the formation of chlorophyll is a complex process to which obviously contributes to some extent manganese ions, but also other trace elements such as potassium, phosphorus and molybdenum. All three compounds studied have proven effective in the process of plant development, and the results highlight the importance of manganese and other elements involved in the direct or indirect process of chlorophyll formation.

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# ANALYSIS OF THE THERAPEUTIC WATER RESOURCES FROM TINCA

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***Abstract:*** *Flow variations of springs can influence the physico-chemical composition of waters and their temperature. Monitoring of the chemical composition is indicated to prevent adverse effects that may occur: formation of compounds with toxic potential, variation of the concentration of components that might lead to deposition and corrosion processes which fill up the pipe. In this paper were performed analysis which allowed a classification of natural waters from Tinca and were compared to earlier results in order to assess the stability of the chemical composition over time.*
***Keywords: AAS methods, IC analysis, therapeutic waters****.*

## INTRODUCTION

Water is a fundamental constituent of life and is essential to a wide range of economic activities. It is also a limited resource. Besides the necessary quality control tools developed for various types of physical, chemical and biological measurements, there is a strong need for education and training related to water quality measurements[11].

Groundwater is the most abundant source of readily available freshwater in the world making up 97% of all freshwater. Due to the slow movement of groundwater through the ground and the very long residence times, the chemical composition of water surface may suffer changes in time [5,7].

Groundwater supports ecosystems and contributes to the achievement of surface water ecological objectives[6].

A good evidence for groundwater can be obtained from a variety of sources, but fundamental to the evidence base is having effective groundwater chemical monitoring[8,9].

Generally, groundwaters are richer in mineral salts than surface waters.

The town Tinca located in the southwestern part of Bihor county, on the right side of Crișul Negru river, at an altitude of 131 m has been known for its waters with therapeutic properties[1]. By the use of these natural waters, which have artesian flowrate, gynecological diseases were treated and also digestive tract problems as: hyperacid gastritis, gastric and duodenal ulcers, chronic colitis, hepato-biliary disorders as biliary dyskinesia, hepatitis, pancreatitis, as well as kidney and urinary tract disorders.

The present paper aims to evaluate the composition of three natural water sources, in order to chemically characterize them.

## EXPERIMENTAL METHODS

The methods of analysis were used according to the specificity of the components.[10] The determination of the anions present in the waters were performed by ion chromatography [2,3] and by potentiometric titration[3,10] and

the analysis of the cations were performed by atomic absorption spectrometry[4].

To carry out this study, water samples were taken from three sources in Tinca: the old well (well_1), well_2 and the external well (well_3).

Samples for pH and volatile constituents were collected untreated. Samples for analysis of cations were filtered and acidified by concentrated superclean $HNO_3$ and samples for anions analysis were filtered through a 0.45 μm membrane filter. The pH was measured electrometric. The bicarbonate content was obtained by potentiometric titration method. The equipment is presented in Figure 1.

*Figure 1. The TitroLine titrator used.*

Chloride, fluoride, phosphate, bromide, sulphate, nitrate and nitrite were analysed by a Ion Chromatograph, type DIONEX AQUION[13]. The eluent solution was prepared with a concentration of 4.5 mM $Na_2CO_3$ and 1.4 mM $NaHCO_3$. Standard solutions of $F^-$, $Cl^-$, $Br^-$, $SO_4^{2-}$, $PO_4^{3-}$, $NO_3^-$, $NO_2^-$ were prepared, starting by standard solutions for ion chromatography of 1000 mg/l from SIGMA-ALDRICH. First were done the calibration curves.

Anions from a small volume of sample are separated by means of a guard column, a separator column and a suppressor column. Anions were determined using a conductivity detector.

The filtered and acidified water samples were analysed by Atomic Absorption Spectroscopy (AAS: PinAAcle 900T), by direct aspiration of the samples into an oxidizing air-acetylene flame for sodium, potassium, magnesium and calcium[12]. Absorption was read at 589.6 nm, at 766.5 nm, at 285.2 nm, respectively at 422.7 nm.

The calibration curves were obtained for standard solutions of each cation prepared from initial solutions of 1000 mg/l, by successive dilutions, according to the recommended highest concentration for respecting the Lambert-Beer law linearity.

A working standard was prepared by diluting the stock solution of 1000 mg$Na^+$/l to 10 mg$Na^+$/l with ultrapure water and then were done five standard solutions: 0.1; 0.2; 0.3; 0.4 and 0.5 mg $Na^+$/l. For potassium analysis, first it was prepared a working standard of 10 mg$K^+$/l and then were done six standard solutions: 0.1; 0.15; 0.2; 0.3; 0.4 and 0.6 mg $K^+$/l. For magnesium analysis was prepared the working standard of 10 mg $Mg^{2+}$/l and from this were done five standard solutions: 0.05; 0.1; 0.15; 0.2 and 0.25 mg $Mg^{2+}$/l. For calcium, the working solution was of 100 mg$Ca^{2+}$/l. From this solution were prepared five standard solutions: 1, 2, 3, 4 and 5 mg$Ca^{2+}$/l.

In order to get the cations analysis by atomic absorption spectroscopy, each series of standards for an element were run and the calibration curves were constructed by plotting the concentrations of the standards against the absorption.

The correlation coefficient of calibration curve was more than 0.99 for sodium, more than 0,998 for

potassium and magnesium and more than 0.995 for calcium.

The chromatograms obtained for each water sample are shown in the figures 2-4.

## RESULTS AND DISCUSSION

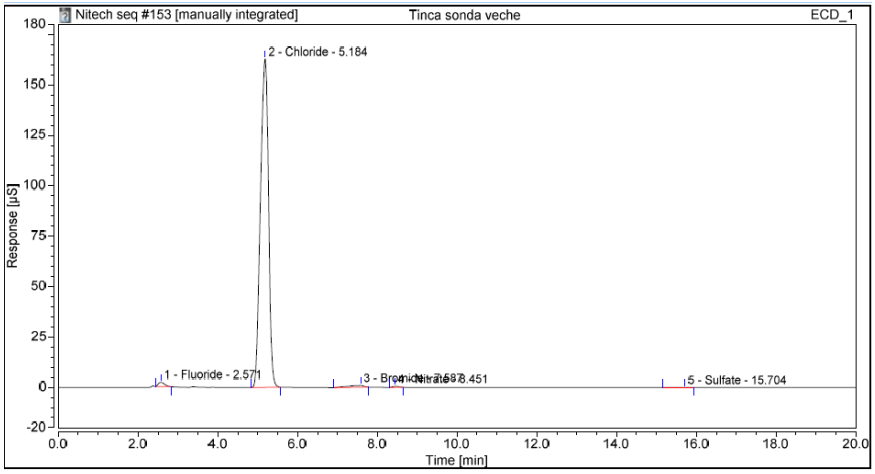


*Figure 2. Chromatogram of the water sample from well_1*

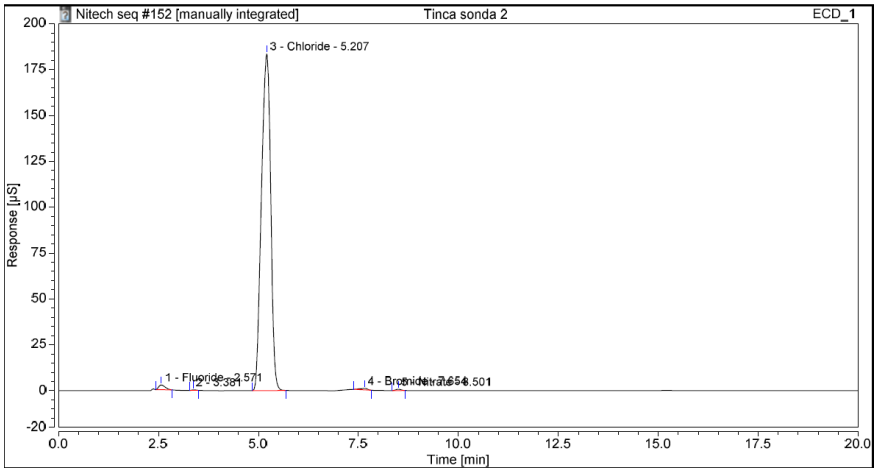


*Figure 3. Chromatogram of the water sample from well_2*

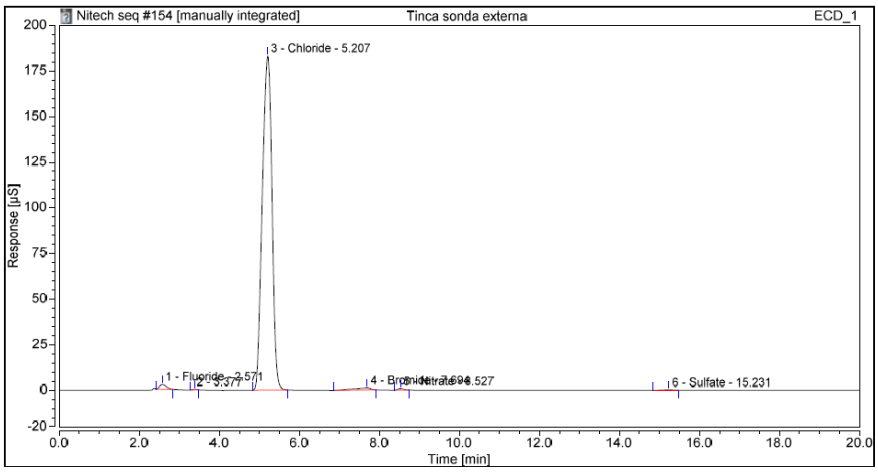


*Figure 4. Chromatogram of the water sample from well_3*

According with the peak area, each concentration of anions present in the water samples were determined, the results being given in tables 1-3.
The dominant anion analysed by chromatography is chloride in waters from all the three sources. The results of potentiometric titration (Table 4) indicated very high bicarbonate concentrations in all analysed samples, being more than 3 g/l.

Table 1. Chromatographic results for well_1.

| Integration Results | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Peak Name | Retention Time min | Area µS*min | Height µS | Relative Area % | Relative Height % | Amount mg/l |
| 1 | Fluoride | 2.571 | 0.379 | 2.070 | 0.97 | 1.24 | 1.7980 |
| 2 | Chloride | 5.184 | 38.123 | 162.896 | 97.71 | 97.87 | 209.7053 |
| n.a. | Nitrite | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 3 | Bromide | 7.587 | 0.422 | 0.910 | 1.08 | 0.55 | 8.0949 |
| 4 | Nitrate | 8.451 | 0.087 | 0.546 | 0.22 | 0.33 | 1.6074 |
| n.a. | Phosphate | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 5 | Sulfate | 15.704 | 0.007 | 0.014 | 0.02 | 0.01 | 0.6713 |

Table 2. Chromatographic results for well_2.

| Integration Results | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Peak Name | Retention Time min | Area µS*min | Height µS | Relative Area % | Relative Height % | Amount mg/l |
| 1 | Fluoride | 2.571 | 0.488 | 2.630 | 0.98 | 1.40 | 2.2947 |
| 2 | | 3.381 | 0.041 | 0.335 | 0.08 | 0.18 | n.a. |
| 3 | Chloride | 5.207 | 49.184 | 183.496 | 98.38 | 97.63 | 270.2775 |
| n.a. | Nitrite | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 4 | Bromide | 7.654 | 0.174 | 0.812 | 0.35 | 0.43 | 3.1662 |
| 5 | Nitrate | 8.501 | 0.105 | 0.679 | 0.21 | 0.36 | 1.8207 |
| n.a. | Phosphate | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| n.a. | Sulfate | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |

Table 3. Chromatographic results for well_3.

| Integration Results | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Peak Name | Retention Time min | Area µS*min | Height µS | Relative Area % | Relative Height % | Amount mg/l |
| 1 | Fluoride | 2.571 | 0.511 | 2.753 | 0.97 | 1.46 | 2.3977 |
| 2 | | 3.377 | 0.040 | 0.332 | 0.08 | 0.18 | n.a. |
| 3 | Chloride | 5.207 | 51.139 | 183.311 | 97.52 | 97.18 | 280.9853 |
| n.a. | Nitrite | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 4 | Bromide | 7.694 | 0.567 | 1.251 | 1.08 | 0.66 | 11.0012 |
| 5 | Nitrate | 8.527 | 0.132 | 0.805 | 0.25 | 0.43 | 2.1430 |
| n.a. | Phosphate | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 6 | Sulfate | 15.231 | 0.052 | 0.170 | 0.10 | 0.09 | 1.0687 |

Table 4. Results obtained at TitroLine

| Well | pH | $HCO_3^-$ mg/l |
|---|---|---|
| Well 1 | 7,19 | 3181 |
| Well 2 | 7,11 | 3505 |
| Well 3 | 7,19 | 3700 |

For getting the cations concentrations in the waters, the samples were aspirated into atomic absorption spectrometer and the absorptions were read at the specific wavelengths for each element. If the measured absorption values of the samples were more than the absorption of the most concentrated standard, the water samples were diluted. Concentrations presented in table 5 were determined from the calibration curves and taking into account the dilution factor.

Table 5. Major cations in water samples

| Well | Concentration, mg/l | | | |
|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ |
| Well 1 | 863 | 66,5 | 40,6 | 117,5 |
| Well 2 | 720 | 205,5 | 44,9 | 105,8 |
| Well 3 | 709 | 51,0 | 45,9 | 110,1 |

## CONCLUSIONS

Physico-chemical analyzes performed on water samples taken from well_2, the outer well - well_3 and the old well - well_1 indicated that these waters have a close neutral pH. From the point of view of the majority ions, the studied waters can be classified as bicarbonate-sodium waters. Among the cations, the sodium concentrations at wells 1 and 3 are very close, around 700 mg / l, and at well_2 the sodium concentration exceeds 850 mg / l. The concentrations of calcium and magnesium ions are comparable in all three sources, those of calcium being around 110 mg / l, and those of magnesium around 40 mg / l. In the case of potassium, a higher concentration was observed in the waters of well_2.

Referring to anions, it can be noticed that the concentrations of bicarbonate ions are very high, exceeding 3 g / l. Chloride ion concentrations are relatively high, ranging from 200 to 280 mg / l. Sulphate ions are present in very low concentrations, in traces, in the waters of the wells 1 and 3 and in sample 2 they were not registered. Taking into account the potability regulations, the concentrations of nitrate ions fall within the potability limit. Among the inorganic anions, the concentrations of fluoride and bromide are higher than those provided in the maximum potability limits, which is why it is recommended to use these waters rationally, for therapeutic purposes, in progressive doses. The presence of phosphate and nitrite ions was not detected, which is beneficial in terms of their quality for drinking purposes.

Compared with old analyzes, it was found that over time, the major ions remained the same, but in the waters from sample 2 the concentrations of magnesium, calcium, sulfate ions decreased and the potassium concentration increased. In water from well 3, among the inorganic anions, the concentration of sulfate ions decreased and the concentration of bromine ions increased, and regarding the cations, there was a significant increase in the concentration of potassium ions. This fact can also be explained by the climatic changes, which led to the change of the water-rock contact time.

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(12pt)

# INSTRUCTIONS FOR AUTHORS (TIMES 14 PT BOLD, CAPITAL LETTERS, CENTRED)

(12pt)

**First name SURNAME[1], First name SURNAME[2] (12 pt bold)**

[1]Affiliations and addresses (12 pt)

(12pt)

***Abstract:*** *Abstract of 50-120 words (12 pt italic). It contains concise information about: objectives of the work, the results obtained, conclusions*

***Key words: List 2-6 keywords. (l0 pt, italic)***.

(12pt)

## INTRODUCTION (l2PT. CAPITAL, BOLD)

The paper has to be written in English. Each paper should be concise including text, figures and tables. Authors are kindly requested to submit in electronic format, Microsoft Word file form, 2003, 2007, 2010. The suggested structure of the main text: Introduction; Methods and Materials, Results and Discussions; Conclusions; References. (12pt)

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Font style: Times New Roman.Text: l2pt.: regular, text in tables: 10 pt, 1 line space and centred, 2 columns, Equations: Equation editor, 12 pt, centred,References (12pt)

c*aption of tables and figures: 12 pt, italic*

Tables, together with figures should be placed in their order of appearance in the text and numbered consecutively. Table captions containing the number of the table, and should be placed above the table. Tables should be clearly captioned and all symbols should be properly explained in either the table or its caption.

Figures (min. 300 dpi) can be in colour, but must also be clear enough for black and white reproduction. They should be centred and numbered consecutively and so referred to in the text. Each must be clearly captioned (after the Figure number) below the figure. Equations will be centred and numbered consecutively (right aligned).

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[1] Abbott, M. B., Petersen, M. M., and Skovgaard, O. (1978). On the numerical modelling of short waves in shallow water, Jnl Hydraulic Res; Vol 16 (3), pp. 23-44.
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