

Keywords: Chitosan, Cysteine, Mercury, Adsorption, Langmuir isothermal, Freundlich isotherms

The present study put an emphasis on the handling of mercury waste by using modified chitosan which obtained from shrimp shell as waste of frozen shrimp. The main aim of the study was to find out the effectiveness of cysteine as modifier to chitosan and its application for adsorption of mercury metal at water bodies. The characterisation study of adsorbent, chitosan-cysteine was done using FT-IR and SEM-EDX, wheres maximum adsorption limit was determined by AAS. The effects of parameters like pH, contact time, maximum adsoption capacity was studied at isothermal condition, on the adsorption process under study. Mechanism of adsorption process was studied according to adsoption kinetic model based under pseudo-first order and pseudo-second order, wheres adsorption isothermal was determined based on Langmuir and Freundlich isotherms. The results of the study indicate that the increase of cysteine amount in the synthetic process has no significant effect on the acquisition of % yield. Adsorption of synthetic results were determined with FTIR, SEM-EDX. The optimum condition of metal ion Hg (II) adsorption by chitosan-cysteine at pH 5.5 with time of adsorption four hours. Kinetics model of adsorption follows pseudo-second order. Data on isothermal adsorption follows Langmuir isothermal model with maximum adsorption capacity to metal ion Hg (II) is 4.99 mmol/g, higher than that of earlier results that is 2.41 m mol g<sup>-1</sup>.

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# Introduction

Shrimp is a reliable commodity of Indonesia in fishing industry which is normally exported in frozen conditions. The shrimp production potential in Indonesia increasing year by year. The national shrimp production in 2011 was 412.000 tons,1 out of which 288.000 tons (about 70%) are exported frozen shrimp and 12.950 tons (about 5%) exported to South Sulawesi. This gives the national shrimp export fifures in last year.

During the process of shrimp freezing, about 25% of the shrimp go waste (skin and head) therefore it is assumed that last year waste produced was 70.000 tons nationally. Such amount of waste should be handled properly to avoid the negative effects on the environment. Since the use of shrimp shell waste is limited only to produce shrimp paste and cattle feed for ducks. Basically the waste has high economic value due to the presence of high percentage of chitin compound (about 20-30%) which also depends upon the types of species.2 One of its uses are as biosorbent to mercury metal as pollutant, which is toxic in nature to the environment.

Several researchers have done modification to chitosan to enhance its mercury adsorption capacities. Among others are oxiran Crosslinked Cd- Complexed Chitosan (2.6 mmol/g),3 chitosan from lobster shell (2.14 mmol/g)4 and aminated chitosan bead (2.26 mmol/g).5 Modification to chitosan by adding cross binding agent is relatively expensive and in general it is toxic, therefore, it is the need of time to improve the ability of chitosan as adsorbent of heavy metal.

In this study L-Cysteine is used as modifier for improving the absorption capacity of Chitosan to mercury metal Hg (II), by making derivative of chitosan, as chitosan-Cysteine. The existence of amine (-NH2) and thyol (-SH) of Cysteine put into chitosan derivative having higher adsorption capacity than chitosan alone. It can function as universal adsorbent to adsorb metal, both soft and hard according to Pearson classification.6

The aim of the study was to find out the effectiveness of Cysteine as modificator to chitosan and its application to adsorb mercury metal in water environment. The effects of parameters like pH, contact time, maximum adsoption capacity was studied at isothermal condition, on the adsorption process under study.. The adsorption process mechanism was studied based on kinetic model of adsorption based on pseudo-first-order and pseudo-second order, whereas isothermal adsorption was determined based on Langmuir and Freundlich isotherms.

# **Materials and Methods**

The materials used in this study were chitosan from Sigma (DD-80%), L-cystein, tetrahydrofuran, NaOH, HCl, HNO<sub>3</sub>, sulfate acid, and acetic acid, KI, KIO<sub>3</sub>, HgCl<sub>2</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

# Preparation of cysteine-chitosan adsorbent

Five (5) grams of L-cysteine was dissolved in 50 mL of tetrahydrofuran and sulfuric acid as catalyst, then added to 5 grams of chitosan which was dissolved in acetate 1% with stirring. The amount chitosan and cysteine varied at a weight ratio of 1:1, 1:2; 1:3, and 1:4 respectively. Synthesis process was followed for 48 hours at a temperature of 50°C and 80°C with stirring by a magnetic stirer. The resulting product is neutralized with aquabidest, filtered and washed until neutral. Then it washed with ethanol to eliminate the excess L-cysteine, and then filtered and dried in a freezing

drier. The resultant product obtained in the form of adsorbent stored in dry and dark containers avoid light and moisture for the further characterized by FTIR and SEM-EDS and for the study of Hg (II) adsorption.

#### Measurement of sulfhydryl group content

The content of the sulfhydryl group was determined by iodimetry. The procedure is as follows: 0.25 g cysteinechitosan was taken in a 50-mL iodine flask and than add 15 mL distilled water, 2.5 mL acetic acid, and 0.5 g potassium iodide. When potassium iodide was completely dissolved, 10 mL potassium iodate (0.003 mol/L) was added. The flask was then put in ice water for 5 min. Then the I<sub>2</sub> produced was titrated with 0.03 mol/L sodium thisulfate as a volumetric solution until the yellow color disappeared. The content of the sulfhydryl group was calculated by the following equation (1) and compared with elemental data analysis, EDX.

$$SH(\%) = \frac{6 \times 10 - \frac{M_2 V_2}{M_1} M_1 \times 33.07}{W \times 10} \qquad \dots \qquad (1)$$

where  $M_1$ ,  $M_2$  are the concentration of KIO<sub>3</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, respectively (mol L<sup>-1</sup>);  $V_2$  is the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (mL); and *W* is the weight of cysteine-chitosan (g).

#### **Adsorption Studies**

# Effect of pH

100 mL of metal ion solution  $Hg^{2+}$  at pH 4-8, 1.0 g cysteinechitosan adsorbent each and stirred at room temperature for maximum 2 hours. Then keep it at rest for 1 hour, then filter with Whatman 42 filter paper. The concentration of metal ion before and after adsorption was determined by AAS. Each experiment was repeated twice. The control was done as earlier except without adsorbent and without stirring.

#### Effect of contact time

100 mL of metal ion  $Hg^{+2}$  plus cysteine-chitosan adsorbent and stirred at room temperature for 2-8 hours. Then keep it at rest for 1 hour, then filter it with Whatman 42 filter paper. The concentration of metal ion before and after adsorption was determined by AAS. Each experiment was repeated twice. The control was done as earlier except without adsorbent and without stirring.

# Adsorption capacity

Solution of metal ion  $Hg^{+2}$  with concentration 50-1000 ppm was prepared at optimum pH. Put into each 100 ml of the solution 1 g cysteine-chitosan adsorbent and stirred at room temperature for an optimum time. Then let it left for 1 hour, then filter it with Whatman 42 filter paper. Concentration of metal ion before and after adsorption was determined by ASS. Each experiment was repeated twice.. The control was done as earlier except without adsorbent and without stirring. The adsorption capacity was calculated by the equation:

$$Q = \frac{V(C_0 - C_e)}{W} \tag{2}$$

in which V is volume of metal ion solution, W is weight of adsorbent (g),  $C_0$  is concentration of metal ion before adsorption (mg/L),  $C_e$  is concentration of metal ion after adsorption (mg/L), Q is capacity of adsorption (mg/g).

# **Result and Discussion**

### Stage of Adsorbent Cystein-Chitosan Making

Adsorbent chitosan modified derivative, cysteine-chitosan was done by the reaction of chitosan and cysteine at the ratio 1: 2; 1: 3; 1: 4, whereas the effect of temperature was observed at 50°C and 80°C for 24 hours. The variation of comparison between chitosan and cysteine was meant to do a reaction of cysteine with chitosan with the amount of surplus cysteine so that to balance reaction to the formation of product, namely chitosan-cysteine (Scheme 1).



Scheme 1. The formation reaction of cysteine-chitosan

The percentage yield from cysteine-chitosan product of synthetic result are given in Fig.1, whereas sulphydrylcontent in adsorbent cysteine-chitosan product was determined by iodometry titration method are in Fig.2. The increase of cysteine amount in synthesis process did not give significant result with the acquisition % yield (Fig.1). The result of the study indicate that the comparison 1:1 saturation reaction is achieved so that with similar condition process, the increase of cysteine amount in reaction given a significant effect in the acquisition of cysteine-chitosan adsorbent product. Therefore, the effect of temperature was also studied in this study, because in order to increase the reaction rate, greater energy is needed. However the result obtained shows that the increase of temperature up to 80°C. did not increase the acquisition of % yield even it tends to decrease (Fig. 2). This is due to temperature above 50°C can cause degradation at chitosan biopolymer, so that the % yield decreased.

The result of this study which is the result of analysis sulphydrylcontent in chitosan-cysteine adsorbent of synthesis showing that the increase of temperature in the process of cysteine-chitosan adsorbent tends to decrease the sulphydryl content in the product (Fig.2).







Figure 2. Content of sulfhydryl groups in cysteine-chitosan

# Characterization of Chitosan-Cystain Adsorbent

# Fourier Transformed infrared spectroscopy (FTIR)

Data on FTIR spectra can be used to identify functions in cysteine-chitosan adsorbent of synthetic result. The characteristic peaks for chitosan FTIR spectra is the peak at 3429-3442 cm<sup>-1</sup> in the form of sharp band and width showing the existence of -OH stretching, weak adsorption at 2.881,65cm-1 and 2.922,16 cm<sup>-1</sup> indicating the existence of -CH stretching 1.649 cm<sup>-1</sup> 1.597 cm<sup>-1</sup> due to amida I and amida II at 1153 cm<sup>-1</sup> indicating stretching of -CN; 1080 cm<sup>-1</sup> indicating stretching -C-O.

The FTIR spectra of cysteine-chitosan adsorbent of synthetic result where new band appears at 2.590 cm<sup>-1</sup> indicating the existence of -SH, carbonyl ester (-CO) appears at the area 1622 cm<sup>-1</sup>; adsorption -CN appears at 1.296- 1.408 cm<sup>-1</sup>; adsorption -CS appears at 1.114- 1.134 cm<sup>-1</sup>.

# Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDX)

Samples of morphology and tophology tested with SEM at Working Distance (WD) 7 mm with HV 20 kV shows property (Fig.3) as follows: chitosan-cysteine sample of synthetic result has homogenous, smooth and thin surface. At the magnification of 20.000 times several cylindrical pores were found with smaller size from  $1\mu m$ .



Figure 3. Micrograf SEM of chitosan (a, b) and cysteine-chitosan (c,d)

Chitosan sample has homogenous rough surface, curved and unfixed pores. The elemnetal analysis done with Energy Dispersive Spectroscopy (EDS) with exitation source of Xray at HV 20kV with WD 15 mm. At cchitosan-Cysteine adsorbent of synthetic result, C,O,N and S components were found, whereas at chitosan only C,O and N components were found. The EDS chitosan-Cysteine spectrum and chitosan is shown in Fig. 3 and in the supplementary material.

# Study of Adsorption Optimum Condition and kinetics of Cysteine-Chitosan Adsorption toward Metal Ion Hg(II)

# Effect of pH

The level of acidity (pH) of solution is an important parameter fully affecting the metal biosorption, surface charge of adsorbent, ionization level and speciation of adsorbate.

In this study the optimization of adsorption process (Hg(II) was varied with pH metal solution at pH 4-8 with early concentration, contact time and constant temperature.

The increase pH increases the amount of metal Hg(II) adsorbed until it reached to an optimum condition at pH 5.5 for chitosan-cysteine adsorption and pH 6.0 for chitosan, then decreased above the optimum pH. This illustrates that the effectiveness of pure ion Hg(II) adsorption process by adsorbent was fully affected by pH of the solution. The denial of electrostatic property between adsorbent active sites of positive charge with metal ion Hg(II) adsorption low below the optimum pH condition.

This is also caused by competition between metal ion Hg (II) and ion  $H^+$  in competing for active adsorption sites, so that its adsorption capacity becomes weaker and weaker. The increase of the pH decreases the protonation level of adsorbent functional groups and the partial positive charges, which increases the adsorption, however, above to this pH value, the adsorbed Hg(II) starts to settle as Hg(OH)<sub>2</sub> precipitated due to reaching the equilibrium hydroxide ion concentration determined by the Ksp value. This hampered the interaction of adsorbate and adsorbent causing the adsorption capacity decresed toward mercury metal.<sup>7</sup>

# Effect of contact time

The result of the effect of contact time on the amount of metal ion Hg (II) adsorbed by chitosan and cysteine-chitosan is shown in Fig. 5.



Figure 5. Effect of contact time at adsorption of ions Hg (II) onto cysteine-chitosan (CC) and chitosan

Fig. 5 shows that the increase of time, amount of ion adsorbed until the balance reached in which the amount of ion adsorbed tends to be constant due to the adsorbent surface has been saturated by metal ion. The results of the study indicate that optimum time of metal ion Hg (II) adsorption by chitosan is 5 hours and for cysteine-chitosan 4 hours. The balance adsorption of metal ion Hg(II) by chitosan-Cysteine absorbent is quicker to be achieved compared with chitosan. The availability of greater functions at chitosan-Cysteine adsorption process completes rapidly (Scheme 2). The time for adsorption balance is one of the important parameters for the economic method of waste water management.

Kinetic model of adsorption based on as pseudo-first order (equation 3) and pseudo-second order (equation 4) is as follows:

$$\lg(q_e - q_t) = \lg \frac{k_1}{2.303} t q_e$$
(3)

$$\frac{t}{q_e} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$$
(4)

These equations are used to analyze mechanism of adsorption comprising movemet of mass and chemical reaction process.

Information on kinetics of metal adsorption is needed to choose optimum condition for the process of metal ion adsorption of batch scale.



Scheme 2. The mechanism of binding of metal ions Hg (II) onto chitosan-cysteine.

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The linear form of the quasi first-order model for the adsorption of ion Hg (II) at chitosan and chitosan-cysteine has shown in (Fig. 6).



**Figure 6.** Pseudo-first order sorption kinetics of Hg(II) onto (a) Cysteine-Chitosan (b) Chitosan

The results of calculation based on equation of quasi order one indicate that coefficient correlation value is low for both chitosan and cysteine-chitosan. Besides that qe value obtained by this method is different significantly with the value of experiment, so that the reaction cannot be classified as pseudo-first order. The parameter of kinetics adsorption based on similarity of pseudo-first order and pseudo-second order is shown in Table 1. The linear form of pseudo-second order is shown in Fig. 7.



**Figure 7.** Pseudo-second order sorption kinetics of Hg(II) onto (a) cysteine-chitosan (b) chitosan

The results of the calculation of the data indicate the knetic adsorption of Hg (II) follows pseudo-second order marked by the value of coefficient correlation  $R^2$ ) close to one for chitosan-Cysteine. Besides that the qe value obtained by this method is not different significantly with the experimental value This shows the occurence of chemosorption involving the attracted power of metal atomic valence and functions through complexity of cysteine-chitosan with metal ion Hg (II). Several studies of kinetic adsorption of metal ion Hg (II) at various adsorbents have reported higher correlation for kinetics model of pseudo-second order.<sup>8</sup>

# Isothermal Adsorption of Metal Ion Hg(II) by Cysteine-Chitosan

Study of isothermal adsorption is important to determine the effectiveness of an adsorption process. In this study adsorption models of Langmuir and Freundlich were used to determine adsorption model between metal ion Hg (II) and adsorbent. Isothermal constants for both models obtained by linear regression calculation based on equation (4) and (7) is shown in Fig. 8, Table 2 and Fig. 9.



**Figure 8.** Langmuir isothermal for the adsorption Hg(II) ions on : (a) cysteine-chitosan (b) chitosan



**Figure 9.**Freundlich Isotermal for the adsorption Hg(II) ions on: (a) cysteine-chitosan (b) chitosan

Langmuir model assumes that adsorption of metal ion occurs at homogenous surface with monolayers adsorption without reaction between adsorbed ion. In order to get balance data, early concentration of metal ion was varied, whereas adsorbent mass in each sample was made constant. The Langmuir isotherm equation is as follows:

$$\frac{c_e}{q_e} = \frac{bc_e}{K_L} + \frac{1}{K_L} \tag{5}$$

$$q_{\max} = \frac{K_L}{b} \tag{6}$$

The calculation result indicates a better correlation with Langmuir isotherm model with the value of  $R^2$  close to 1, both for adsorbent chitosan-Cysteine ( $R^2 = 0.972$ ) and chitosan ( $R^2 = 0.970$ ). The closeness between adsorbate and adsorbent can be predicted by using Langmuir parameter that is separation factor, R<sub>L</sub>:

$$R_L = \frac{1}{1 + bC_0} \tag{7}$$

in which  $C_0$  is early concentration of metal ion and *b* is constant of Langmuir isothermal.  $R_L>1$  is not beneficial,  $R_L=1$  linear,  $0 < R_L < 1$  is beneficial,  $R_L=0$  Irreversible.

This indicates the balance of isotherm is very compatible with Langmuir model and adsorption process is monolayer adsorption to the surface of adsorbent with the amount of identical limited sites, distributed homogenously through the surface of adsorbent.

Freundlich isotherm model with the assumption that the adsorption of monolayer and process of adsorption occur at the heterogenous surface with adsorption capacity and concentration of Hg (II) at balance. The Freundlich isotherm equation is as follows:

$$\lg q_e = \lg K_F + \frac{1}{n} \lg C_e \tag{8}$$

 $K_{\rm F}$  is relative adsorption of capacity and n is size of characteristic and strength of the adsorption process and distribution of active sites. The result of calculation shows low correlation with Freundlich isotherm model indicated by the value of R<sup>2</sup> < 0.99, both for chitosan-Cysteine adsorbent ( $R^2 = 0.925$ ) and chitosan ( $R^2 = 0.795$ ). The value of n based on mathematical calculations between 1 and 10 indicates effective adsorption.<sup>9</sup>

**Table 1.** Kinetic parameters obtained from Lagrangian models in the adsorption of Hg (II) onto cysteine-chitosan (CC) and chitosan ( $C_0 = 30$  ppm,  $w_{ads} = 0.1$  g, volume = 0.1 L)

	Pseudo-firs	t order			Pseudo-second-order				
Adsorbent	Adsorbent $K_1(h^{-1}) = q_e (mg g^{-1})$		)	$R^2$	$K_2 (g mg^{-1} h^{-1})$	$q_{\rm e}~({\rm mg/g})$		$R^2$	
		Calcd.	Exp.			Calcd.	Exp.		
CC	0,6541	15,3462	27,5417	0,766	0,245	28,5714	27,5417	0,997	
Chitosan	0,4514	6,8865	6,8865         23,7667         0		0,882	23,8095	23,7667	0,999	

Table 2. Adsorption equilibrium constants obtained from Langmuir and Freundlich isotherms in the adsorption of Hg(II) onto cysteinechitosan (CC) chitosan

	Langmuir 1	nodel		Freundlich model							
Adsorbent	$q_{max}$ (mg g <sup>-1</sup> )		Kl,	B, dm <sup>3</sup> mg <sup>-1</sup>	RL	$\mathbb{R}^2$	$q_{\rm max}$ , mg g <sup>-1</sup>	п	KF	$R^2$	
	Calcd.	llcd. Exp. d		m <sup>3</sup> g <sup>-1</sup>			Calcd.				
CC	1000,0068	483,5414	6,4103	0,0064	0,3184	0,972	813,1152	1,6447	15,1008	0,925	
Chitosan	500	331,4083	4,3859	0,0088	0,2068	0,970	422,1502	1,8904	14,2889	0,795	

# Conclusion

The resuls of this study indicate that the effectiveness of cysteine as modificator to chitosan is at the comparison of mol 1:3. pH solution of metal ion and time of adsorption fully affect the adsorption capacity of cysteine-chitosan. The balance of metal ion Hg (II) adsorption by cysteine-chitosan is quicker than chitosan that is 4 hours at pH 5.5. Based on the data of the findings, adsorption of metal ion Hg(II) by cysteine-chitosan follows kinetic model of pseudo-second order and Langmuir adsorption isotherm with the capacity of maximum adsorption is 4.99 mmol g<sup>-1</sup>.

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Mixed solid state / solution phase routes were applied to synthesize a new family of fluoride-free synthetic clays for water remediation applications. The selected samples of synthetic fluoride-free–Na-4-micas had the general formula of Na<sub>4</sub>Mg<sub>6</sub>M<sub>4</sub>Si<sub>4</sub>O<sub>22</sub>·nH2O, where  $M = Al^{3+}$ , Bi<sup>3+</sup>, and Cr<sup>3+</sup>. Structural and micro-structural properties were determined by using both XRD and SEM. Determining the grain size of the mica, bulk was found to be in the range of 2.37–3.43 µm, which was lower than those reported in the literature. Electrical property investigation proved that Al-clay and Bi-clay exhibited semi-conducting behaviour, but Cr-clay exhibited insulating behaviour. This latter confirmed that the energy gap  $\Delta E_g$  was the largest for the chromium clay.

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# 1. Introduction

The group of synthetic clays "swelling micas", out of which only one Na-4-mica, were originally developed exclusively for water treatment. They imbibe as they absorb metal ions, then collapse with sealing the metals inside. Na-4-mica is formed by combining kaolinite, a soft clay mineral used in the ceramics industry, with magnesium oxide in sodium fluoride at a temperature of 890°C. The resulting product has sheet like structure (as natural mica's) with brittle composition, but having space between the layers.<sup>1-3</sup>

Waste effluents in mining operations and various chemical processing industries contain heavy metals which are nonbiodegradable and toxic pollutants. Due to their tendency to accumulate in living organisms, causing various diseases and disorders, the treatment methods for metal-containing effluents are essential for environmental and human health protection. Among numerous commonly used techniques for water purification, adsorption techniques have gained much attention because of their cost effectiveness and easy operation.<sup>4-18</sup>

In recent years, an intensive research was conducted focusing on the selection and/or development of low-cost adsorbents with good metal-binding abilities, which could be utilized as an alternative to the most widely used adsorbent, activated carbon in wastewater treatment. Natural materials of both organic and inorganic nature (such as chitosan, zeolites, minerals, etc.) and certain waste products from industrial operations (such as fly ash, coal and oxides) are already classified as low-cost adsorbents because of their local availability and economic nature.<sup>19-28</sup>

The major objective of the present article is to investigate the effect of solution route synthesis on

a) the structural and nano-structural properties of  $Na_4Mg_6M_4Si_4O_{22}$  ·nH<sub>2</sub>O mica clay samples, and on

b) the electrical properties of  $Na_4Mg_6M_4Si_4O_{22}\cdot nH_2O$  mica clay samples.

# 2. Experimental section

# 2.1. Sample preparation

The selected samples of synthetic fluoride-free–Na-4-mica with the general formula of  $Na_4Mg_6M_4Si_4O_{22}$ ·nH<sub>2</sub>O, where  $M = Al^{3+}$ ,  $Bi^{3+}$ , or  $Cr^{3+}$ , were synthesized by applying solution phase route and sintering procedure using the molar ratios of highly pure  $Na_2O \cdot 2SiO_2 \cdot 2H_2O$ ,  $MgCO_3$ ,  $Al_2O_3$ ,  $Bi_2O_3$ , and  $Cr_2O_3$ . Mixtures were grounded carefully, then they were dissolved in concentrated nitric acid to form nitrate extract, which was then diluted with distilled water. The nitrate solution was than neutralized by using 45 % urea solution with pH ~ 6.5.

A sodium silicate solution was prepared first and noted as Mixture I. Another solution, mixture II, contained the required Al-, Mg-, Bi-, and Cr-nitrates according to the desired chemical formula. The pH of Mixture I was adjusted to 8.5, then a concentrated solution of ammonia was added carefully until thick white precipitate of metal hydroxides were obtained, according to the fact that pH must be higher than 8. The precursor is filtered, and washed with 2.5 % ammonium nitrate solution. Mixture II containing Al-, Mg-, Bi-, and Cr-salts was passed through the same treatment but in the presence of ethylene glycol as complexing agent in order to produce gelatinous precipitate of metal cation hydroxide precursors.

#### Fluoride-free silicate based mica-like clays

Precursors, Mixture I + II, were placed into a muffle furnace for calcination process at 880 °C under a compressed air atmosphere for 15 hours, then re-grounded and pressed into pellets (thickness 0.2 cm and diameter 1.2 cm) under 10 Tons /cm<sup>2</sup>. Sintering was carried out under air stream at 1050 °C for 10 hours. Samples were slowly cooled down (20 °C /hour) till 500 °C and annealed there for 5 hours under air stream. The furnace was shut off and cooled slowly down to room temperature. Finally, the product materials were kept in vacuum desiccators over silica gel dryer.

The synthesized samples were named as Clay I  $(Na_4Mg_6Al_4Si_4O_{22}\cdot nH_2O)$ , Clay II  $(Na_4Mg_6Bi_4Si_4O_{22}\cdot nH_2O)$ , and Clay III  $(Na_4Mg_6Cr_4Si_4O_{22}\cdot nH_2O)$ . As shown in Fig. 1, tetrahedral units of silicate forms the backbone of mica clay structure, indicating that each unit cell is surrounded by 4-Na-atoms that can be replaced if it is applied as cation exchanger.



Figure 1. Structure of Al-silicates – Clay I

#### 2.2. Phase identification

The X-ray diffraction (XRD) measurements were carried out at room temperature on fine ground samples using Cu- $K_{\alpha}$  radiation source, Ni-filter, and a computerised STOE diffractometer from Germany with two theta step scan technique.

Scannig Electron Microscopy (SEM) measurements were carried out at different sectors in the prepared samples by using a computerized SEM camera with elemental analyzer unit (PHILIPS-XL 30 ESEM from USA).

### 2.3. Electrical measurement

DC-electrical conductivities of prepared materials were recorded as a function of temperature from room temperature up to 520 °C, and measurements were performed on the pellet surface by using two probe circuit and graphite paste as connective matter.

# 3. Results and discussion

# 3.1. Phase identification

Fig. 2 displays the X-ray powder diffraction patterns recorded for synthetic fluoride-free–Na-4-mica samples with the general formula of  $Na_4Mg_6M_4Si_4O_{22}$ ·nH<sub>2</sub>O, where M =  $Al^{3+}$ ,  $Bi^{3+}$ , and  $Cr^{3+}$ . The analysis of corresponding 2 $\theta$  values and inter-planer spaces d (Å) were carried out using a

computerized program. This latter indicated that the X-ray crystal structure mainly belongs to a monoclinic phase Na<sub>4</sub>Mg<sub>6</sub>M<sub>4</sub>Si<sub>4</sub>O<sub>22</sub>  $\cdot$ nH<sub>2</sub>O in major, besides few peaks of unreacted starting oxides as secondary phase in minor. The lattice parameters of the unit cell were refined using the least-squares sub-routine on a standard computer program. These refined lattice parameters were found typically to those reported in references [19-23]. These unit cell parameters are in good agreement with those reported for Na<sub>4</sub>Mg<sub>6</sub>M<sub>4</sub>Si<sub>4</sub>O<sub>22</sub> nH<sub>2</sub>O.<sup>6</sup>



Figure 2. X-ray diffraction patterns for Al-clay (Clay I, top), Biclay (Clay II, middle), and Cr-clay (Clay III, bottom)

It is obviously that the addition of nano-oxide components has a negligible effect on the main crystalline structure of  $Na_4Mg_6M_4Si_4O_{22}$ ·nH<sub>2</sub>O with fluoride content (x = 0.0) as shown in Fig. 2 (bottom).

From Fig. 2 (top and middle) one can interpret that the monoclinic phase of mica-clay Na<sub>4</sub>Mg<sub>6</sub>M<sub>4</sub>Si<sub>4</sub>O<sub>22</sub>·nH<sub>2</sub>O is the dominating phase, which exceeds 90% ( $d_{100} = 1.12-1.13$  nm), confirming that nano-oxide component are successfully reacted and formed monoclinic biotite phase with a good degree of crystal nature.

EDX analysis indicated that the atomic percentage recorded is a bit typical for the molar ratios of the prepared sample, emphasizing the quality of preparation through solution technique.

On the basis of molar ratio, the allowed error in experimental procedures throughout solution route is smaller than those reported in literatures for samples synthesized by solid state routes.<sup>1,27</sup>

#### 3.2. SE-microscopy measurements

Fig. 3-5 show SEM-micrographs recorded for  $Na_4Mg_6M_4Si_4O_{22} \cdot nH_2O$ , where  $M = Al^{3+}$ ,  $Bi^{3+}$ , and  $Cr^{3+}$ , prepared via solution route. The estimated average of grain size was calculated and found to be in the region of 2.37 – 3.43 µm supporting the data already reported in ref. [23].

Figure 3. SE-micrographs recorded for Al-clay-I with different magnification factors



Figure 4. SE-micrographs recorded for Bi-clay-II with different magnification factors



Figure 5. SE-micrographs recorded for Cr-clay-III with different magnification factors

The EDX examinations for random spots in the same sample confirmed and are also consistent with our XRD analysis for polycrystalline  $Na_4Mg_6M_4Si_4O_{22}$ ·nH<sub>2</sub>O, which was prepared via solution route, so that the differences in the molar ratios estimated by EDX for the same sample is emphasized as an evidence for the existence of monoclinic phase with good approximation to molar ratios.

It is difficult to observe inhomogeneitiy in Fig. 3-5 within the micrograph due to the fact that powders used are very fine and the particle size estimated is too small. This indicates, that the actual grain size in the material bulk is smaller than that detected on the surface morphology. Furthermore, particle size was estimated from both XRD and SEM analyses and its average was found to be between 25 and 124 nm, confiming that solution route synthesis increases the fraction ratio of nano-particle formation.

# **3.3. Electrical properties**

Fig. 6 shows the DC-electrical conductivity of the prepared mica-materials  $Na_4Mg_6M_4Si_4O_{22}\cdot nH_2O$ , which was recorded as a function of temperature from room temperature up to 520 °C, and performed on the pellet surface by using two probe circuit and graphite paste as connective matter.

It is clear that the conduction increases as temperature raises reflecting semiconductor behaviour for both Al-clay-I and Bi-clay-II samples, while insulating behaviour for Crclay-III sample.

Although most of mineral silicate-structure clays in the literature are semiconductors,<sup>22-25</sup> Cr-clay-III was found to has an insulating behaviour through the investigated temperature range, reflecting the larger energy gap for Cr-clay-III.

Transport properties of the materials obeys the Arrhenius equation  $\sigma_{ac} = \sigma_0 \exp(-E_a/K_BT)$ , where the symbols have their usual meanings. It is observed that the conductivity of the material increases with the increase of the temperature, and shows the negative temperature coefficient of resistance behaviour for both clay I and clay II. The calculated values of activation energies of the clay compounds are 0.34, 0.41, and 1.39 eV for Clay I, Clay II, and Clay III, respectively. This behaviour suggests that the conduction mechanism of the investigated compounds may be due to charge carrier which enhanced by cation holes, and the energy gap between the conduction and valence band increases from clay I to clay III.

From Fig. 6 it is clear that Bi-clay-II exhibits semiconducting behaviour up to 410 °C and loose its semiconducting behaviour above this temperature due to an increase in its energy gap, which results in an insulating behaviour as shown in Fig. 6.



Figure 6. DC-electrical conductivity for Clay I, Clay II, and Clay III as a function of absolute temperature

# 4. Conclusion

The conclusive remarks of the present article can be summarized as follows:

1. solution phase technique exhibits structure quality as a preparation technique,

2. synthetic fluoride-free  $Na_4Mg_6M_4Si_4O_{22}\cdot nH_2O$  crystallizes in monoclinic phase,

3. SE-micrographs confirmed that particle size was in nano-scale (25-135 nm),

4. Clay-III (Na<sub>4</sub>Mg<sub>6</sub>M<sub>4</sub>Si<sub>4</sub>O<sub>22</sub> $\cdot$ nH<sub>2</sub>O, where M = Cr<sup>3+</sup>) has the highest Eg value of 1.39 eV.

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Keywords: sour cherry, metal elements, hyperlipidemy.

Sour cherry (*Prunus cerasus*) is a widely favored fruit worldwide. Sporadic studies were done to determine the metal ion content of this fruit. Nevertheless its effect on metal ion homeostasis has not been examined so far therefore, experiments on animal (Wistar rats) were carried out to determine the changes of metal homeostasis in liver by the effect of this fruit. Wistar rats were divided into four groups: 1. control animal with normal diet; 2. hyperlipidemic rats were fed with fat-rich diet (chow contained plus 2% cholesterol, 0.5% cholic acid and 20% sunflower oil); 3. rats were fed with normal diet + lyophilized sour cherry (0.75 g daily ad libitum); 4. rats were fed with fat-rich diet and lyophilized sour cherry. The experiment was terminated after 10 days. From the sample handed rat liver homogenate metal ion content was determined by inductively plasma optical emission spectrometry (ICP-OES). Liver fragments were fixed in 4% neutral buffered formalin, embedded in paraffin, and 5 micrometer sections were cut and stained with hematoxylin–eosin. As a result of our experiment the concentration of metal elements were found to decrease significantly in the hyperlipidemic animals fed with sour cherry (Újfehértói fürtös), although there wasn't any significant change in result between the 1. (control animal with normal diet) and the 3. (rats were fed with normal diet + lyophilized sour cherry) group. On the basis of histological study it was established that the treatment with Fanal was the best, although Pipacs and Újfehértói fürtös were also significant in liver regression of hyperlipidemic animals with fatty liver, therefore it is concluded, that sour cherry treatment is beneficial to lower the hyperlipidemy and fatty degeneration.

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# Introduction

Nowadays it is common that improper nutrition has a deteriorative effect in the redox- and metal homeostasis of the human organism and that can lead to cardiovascular or gastrointestinal diseases and hyperlipidemia also.

Sour cherry (*Prunus cerasus* L.) is one of the most popular fruits in Europe. It could be used as a "functional food", because of its anti-inflammatory, anti-diabetic and lipid lowering properties<sup>1-4</sup>. The bioactive compounds in sour cherry (*Prunus cerasus* L.) also have beneficial effects on cardiovascular diseases<sup>5</sup> and certain types of cancers. Some studies report that its bioactive agents such as anthocyanins (e.g. cyanidin, peonidin, cyanidin-3-Orutinoside) and other polyphenols in *Prunus cerasus* L. have protective effects on the neuron cells too<sup>6</sup>. Results of the investigations revealed the strong antioxidant activity of anthocyanins and their possible use as chemotherapeutics<sup>7-8</sup>. Furthermore it attracted much attention that antochyanins possess anti-inflammatory properties as well<sup>9</sup>. Since in previous studies it was justified that the presence of some transition metal (Fe, Ca, Cu, Mn, Zn) elements has a significant effect on the signal transduction process<sup>10-11</sup>, our aim was to determine the microelements in three types of sour cherry and their biological effects in hyperlipidemic animals.

# Experimental

### Materials

Ketamine (Calypsol) were purchased from Richter Gedeon (Budapest, Hungary) and xylazin (Sedaxylan) were from Eurovet Animal Health BV (Bladel, Hollandia). ICP standars originates from Spectro Ltd. (Kleve, Germany).

### Fruit samples and extraction

The following three types of sour cherry(*Prunus cerasus* L.) were examined: Pipacs 1 (M1), Fanal (M2) and Újfehértói fürtös (M3).

The cherry trees were cultivated at the Research and Extension Center for Fruit Growing in Újfehértó, Hungary. Fruits were harvested in 2011 in the optimal ripening stage and later halved and pipette out. The fresh fruits were lyophilized (Labconco, USA) and stored at  $-80^{\circ}$ C until experiments. The lyophilized sample (1 g) was delivered into a 100 mL centrifuge tube, and 50 mL of MeOH:H<sub>2</sub>O:HCOOH mixture (60:39:1, v/v) were added. The sample was vortexed and after 1 hour of ultrasonic bath, the suspension was filtered. The extract aliquot solvent (10 mL) was evaporated using a rotary evaporator, under vacuum to dryness at a temperature of 40 °C.

# **Animal experiments**

Young male Wistar albino rats (150-200 g body weight) were weighed and randomly divided into four types of groups with 5 animals in each group.

The rats in group 1 were kept on a normal diet obtained from BIOFARM FARM PROMT Kft. (BFP, Gödöllő, Hungary). The rats of the second group were kept on a fatrich diet containing cholesterol (1.0%), sunflower oil (11%) and cholic acid (0.3%) added to the control BFP. The third group was fed with the same normal diet completed with lyophilized sour cherry powder mixed into the diet (0.75 g daily ad libitum). The rats in group 4 were kept on fat-rich diet completed with lyophilized sour cherry powder (0.75 g daily ad libitum). The rats were kept on the diets for 10 days. The animals in group 3 and 4 were divided into three-three other groups correspondently the three types of sour cherry: 'Pipacs 1', 'Fanal' and 'Újfehértói fürtös'.

Finally, the rats were anaesthetised with ketamine (75 mg/kg) and xylazin (7.5 mg/kg). After laparotomy, blood was collected from the abdominal vein and the animals were exsanguinated. Liver was removed, washed and homogenized in ice-cold isotonic KCl solution.

Hyperlipidemy was proved by histological study.

# Histology

Liver fragments were fixed in 4% neutral buffered formalin, impregnated in paraffin, and 5 mm sections were cut and stained with hematoxylin–eosin.

# **Protein measurement**

The protein content of liver homogenate was set at 10 mg/mL using bovine albumin as a standard, which was measured in accordance with the method of Lowry et al.  $(1951)^{12}$ .

# **Determination of metal elements**

The homogenized liver samples (3 g, 5 parallel for each) were digested with heating in 10 mL HNO<sub>3</sub> (65%) and 2 mL  $H_2O_2$  (30%). After all, the solution was filled up to 10 mL with bidistilled water. The element (Al, B, Ba, Ca, Co, Cu, Fe, Li, Mg, Mn, Ni, P, Pb, Sr, Zn) content was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) with Spectro Genesis (Kleve, Germany) appliance<sup>13</sup>.

Statistics

The results are reported in mean values and standard deviation, which were determined by Excel 2010 software programme. Differences between two independent groups of data were analyzed with 2-tailed-t-test. One-way analysis of variance (ANOVA) was used to compare multiple groups. Differences at P < 0.05 were considered significant. Statistical analysis was carried out by Graphpad software version 1.14. ANOVA was calculated for control, control+M1, fat-rich diet, and fat-rich diet +M1; for control, control+M2, fat-rich diet, and fat-rich diet +M2; as well as for control, control+M3, fat-rich diet and fat-rich diet +M3.

### Results

#### Histology

According to the histological studies treatment with Fanal was the most beneficial to lower the hyperlipidemy in animals. Pipacs1 and Újfehértói fürtös were also effective against tissue necrosis but the treatment with Fanal resulted lower quantity of lipid droplets in hepatic cells (Figures 1 and 2).



**Figure 1.** Fatty liver (Arrows show the diffuse hepatocellular degeneration with balloon cell-like hepatocytes in the liver of animals fed with fat-rich diet. Bar scale:  $100 \mu m$ )



Figure 2. Hepatic lobule shows the beneficial effect of Újfehértói fürtös sour cherry treatment in hyperlipidemic animals (Arrow shows the vena centralis. Bar scale: 100  $\mu m)$ 

1.

	Control		Control+N	41	Control	-M2	Control	-M3
	Main	Standard	Main	Standard	Main	Standard	Main	Standard
		deviation		deviation		deviation		deviation
Al	0.388	0.330	1.006	1.629	0.117	0.082	0.479	0.382
В	1.62	1.426	1.511	0.279	0.635	0.416	1.587	0.945
Ba*	0.0351	0.014	0.028	0.003	0.0195	0.006	0.0318	0.013
Ca	2.00	0.85	3.15	1.29	2.03	0.84	1.77	0.66
Co	0.0051	00.001	0.004	0.0001	<dl< td=""><td>-</td><td><dl< td=""><td>-</td></dl<></td></dl<>	-	<dl< td=""><td>-</td></dl<>	-
Cu	0.186	0.019	0.180	0.027	0.175	0.036	0.252	0.101
Fe	6.78	1.37	7.79	1.67	6.26	1.69	8.83	5.83
Li	0.0043	0.0030	0.0023	0.0010	0.0019	0.0010	0.0029	0.0010
Mg	8.52	0.944	8.23	0.954	8.08	1.41	9.37	1.41
Mn	0.102	0.025	0.107	0.023	0.0861	0.017	0.099	0.016
Ni	0.0112	0.006	0.0080	0.0030	0.0073	0.0060	0.0210	0.0190
Р	187.9	18.9	179.4	17.9	169.3	31.3	195.8	28.7
Pb	0.0370	0.0300	0.0258	0.020	0.0282	0.0001	0.0104	0.0001
Sr	0.0053	0.0040	0.0031	0.0010	0.0024	0.0020	0.0025	0.0020
Zn	1.34	0.17	1.44	0.25	1.29	0.28	1.48	0.28

Element concentrations  $(\mu g/g)$  in rat liver of control groups (n=5)

\*significant difference between control and control+M2, M1: Pipacs 1 type sour cherry, M2: Fanal type sour cherry, M3: Újfehértói fürtös type sour cherry,<dl under detection limit

It can be stated that sour cherry consumption indicates protective effect on fatty liver, because lipid droplets appear in less quantity than in the necrotic liver. Supposing the bioactive content is similar in every types of sour cherry; however its quantity is different. It is also considerable that the environmental factors may have some influence on the content quality.

#### Metal elements

Table

The results of control groups are shown in Table 1, while the results of fat-rich diet groups are in Table 2.

According to the results in Table 1, the consumption of Pipacs1 (M1) causes raising tendency in the concentration of Al, Ca, Fe, Mn and Zn in the control group. The ascent of the metal elements with Fanal (M2) was different, because only Ca, Fe and Ni concentrations altered considerably. The most essential changes of metal element concentration occurred by Újfehértói fürtös (M3). Considerable increase was observed in Al, Cu, Fe, Mg, Ni, P and Zn concentrations. The control group shows only one significant difference in the Ba concentration, which is caused by Fanal (M2).

In Table 2 the concentrations of most metal elements, such as Al, B, Cu, Mg, Mn, Ni, P and Zn are raised by the effect of sour cherry Pipacs1 (M1) consumption. Three metal elements (Cu, Mg, Mn) show raising tendency in the group with Fanal (M2), and only two (Al, B) in the group with Újfehértói fürtös. Significant decrease can be apparent in the concentration of Ba, Fe, Sr in fatty liver, which is

brought about by Pipacs1 (M1). Beside that Fanal (M2) results significant reduction in the concentration of B, Ca,

and Sr. The concentrations of Al, Ba, Ca, Fe, Sr are significantly decreased by the effect of Újfehértói fürtös compared to the atherogen group. The results of ANOVA show that the metal element content in the liver is mostly influenced by Újfehértói fürtös (M3).

On average the changes of the metal element concentration between the control and fat-rich diet fed groups, were different by the effect of each of the sour cherry consumption. Compared to the fat-rich diet fed groups, the changes of the metal element concentrations were almost the opposite, except the results of Fanal (M2), which shows almost the same decreasing tendency in the fat-rich diet fed and control groups as well.

# Discussion

On the basis of histological studies it was established that treatment with Fanal was the best, although Pipacs and Újfehértói fürtös were also significant in liver regression of hyperlipidemic animals with fatty liver.

The results also showed that none of the three types of sour cherry change the metal-homeostasis in the control groups. Therefore, it may be considered that, sour cherry don't have a negative effect on the healthy metalhomeostasis.

In conclusion to the result of our experiment all the three types of sour cherry have a positive effect on the metalhomeostasis and its treatment is also beneficial to lower the hyperlipidemy and fatty degeneration.

**Table 2.** Element concentrations  $(\mu g/g)$  in rat liver of atherogen groups (n=5)

	Atheroge	en	Atherog	en+M1	Atheroge	n+M2	Atheroge	n+M3	ANOVA
	Main	Standard	Main	Standard	Main	Standard	Main	Standard	between
		deviation		deviation		deviation		deviation	C, C+MX, A and
									A+MX
Al ****	0.268	0.221	0.437	0.466	0.141	0.0795	0.913	0.395	M3
B ***, ****	0.769	0.266	1.922	1.223	0.358	0.1079	1.3007	0.593	-
Ba*,**,****	0.0371	0.003	0.0278	0.0028	0.0293	0.0211	0.0265	0.007	-
Ca ***,****	2.75	0.799	2.56	1.288	1.49	0.3990	1.66	0.754	-
Co	0.003		<dl< td=""><td></td><td><dl< td=""><td></td><td>0.002</td><td></td><td>-</td></dl<></td></dl<>		<dl< td=""><td></td><td>0.002</td><td></td><td>-</td></dl<>		0.002		-
Cu	0.155	0.029	0.176	0.0250	0.175	0.0289	0.135	0.030	M3
Fe **,****	7.14	1.232	4.51	0.484	5.61	2.26	4.01	1.63	M1
Li	0.0041	0.0040	0.0034	0.0016	0.0031	0.0022	0.0023	0.001	-
Mg	7.92	0.708	8.10	0.569	8.01	0.884	7.0014	1.23	M3
Mn	0.0692	0.0050	0.0714	0.0077	0.0794	0.0167	0.0609	0.014	M1, M3
Ni	0.0069	0.0040	0.0289	0.0257	0.0067	0.0058	<dl< td=""><td><dl< td=""><td>-</td></dl<></td></dl<>	<dl< td=""><td>-</td></dl<>	-
Р	162.2	11.89	167.5	16.71	161.1	17.62	140.4	31.36	M3
Pb	0.0978	0.156	0.0522	0.0025	<dl< td=""><td></td><td><dl< td=""><td></td><td>-</td></dl<></td></dl<>		<dl< td=""><td></td><td>-</td></dl<>		-
Sr **,***,***	0.0063	0.002	0.0034	0.0019	0.0021	0.0019	0.0023	0.001	M3
Zn	1.344	0.326	1.409	0.1809	1.3004	0.1703	1.13	0.277	-

\*\*significant difference between A and A+M1, \*\*\* significant difference between A and A+M2, \*\*\*\* significant difference between A and A+M3; M1: Pipacs 1 type sour cherry, M2: Fanal type sour cherry, M3: Újfehértói fürtös type sour cherry,; <dl under detection limit

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# REVIEW ON THE UTILIZATION OF WAX PHASE CHANGE MATERIALS (WPCMs) IN CHINA

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Keywords: review; wax; phase change; materials; utilization

The utilization of wax phase change materials (WPCMs) as raw material has been reviewed in the present article. Additional use of WPCMs into the solar energy system, the construction system, the air conditioning system and the textiles products has also been discussed. The melting point, fusion heat and the properties of commercial WPCMs have also been compared. The complete utilization of WPCMs has been resulted in good economical and social benefits.

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# **INTRODUCTION**

Presently the utilization of thermal storage capacity of wax phase change materials (WPCMs) is under intense focus because of their high temperature thermal storage capacity, high thermal efficiency and also keeps energy at a constant temperature etc. WPCMs not only change their phase but also absorb or release a lot of latent heat with the change of the reaction temperature.<sup>1</sup> These are having the advantages of high latent heat capacity during the phase change process, no supercooling, low steam pressure during the melting process, no chemical reaction taking place, stable performance, almost no change of phase temperature and phase latent after absorbing or releasing latent heat several times, no phase separation, no corrosive properties and low price also. Therefore, WPCMs are widely used in the different areas such as solar energy, residual industrial heat, waste heat recovery and saving energy during construction etc.<sup>2</sup>

In the present paper, authors have discussed the use of WPCMs in different areas such as the solar energy, the construction, the air conditioning systems and the textiles products have been discussed and a comparison to the melting point, fusion heat and the properties of commercial WPCMs also.

# DISCUSSION

The melting point and fusion heat of commercial wax phase change materials <sup>3</sup> are shown in Table 1. Astorstat is a product coming out from Astor Paraffins/Waxes Honeywell, had number one melting point range. However, Micronal (microcapsule) from BASF had observed a minimum melting point range. On the other hand, Rubitherm RT's fusion heat had the wide range as compared to Astorstat and Micronal (microcapsule), which had no fusion heat range, therefore the properties of Rubitherm RT were found better than that of other products.

Table 1. The	melting	point an	d fusion	heat of	commercial	wax
phase material	s					

Name	Melting	Fusion	Manufacturer
	point (°C)	heat	
		(kJ/kg)	
Astorstat	0-148.9	-	Astor
			Paraffins/Waxes
			Honeywell
Rubitherm RT	-7-100	130-214	Rubitherm GmbH
Rubitherm GR	27-80	64-72	Rubitherm GmbH
(granulate)			
Rubitherm PX	41-82	110-117	Rubitherm GmbH
Micronal PCM	23-26	-	BASF
(microcapsule)			
Outlast	6-79	160-200	Outlast
Thermocules			
(microcapsule)			

### The properties of wax phase change material <sup>4</sup>

Table 2 shows the heat properties of n-paraffins. The melting point of even-numbered n-paraffins increased with an increase in the molecular weight. Further it has been noticed that, evennumbered n-paraffins had higher melting heat than that of oddnumbered n-paraffins. However, odd-numbered n-paraffins did not follow any rule with the molecular weight.

# Utilization of wax phase change materials in the solar energy system

The energy storage for solar power as a clean and unconventional energy source is widely studied and used. Its phase change storage has large developing markets because of high temperature thermal storage capacity, maintaining at a constant temperature. Wax used in the solar heating water system and the solar air conditioning system is one of the phase change materials. The solar heating water system with phase change materials transfers heat energy from cold water heated during daytime to wax phase change materials. Its storage energy is kept through the melting process. Phase change materials release energy to cold water during night. This is one cycle system.<sup>5</sup> Feng Xiaojiang <sup>6</sup> introduced a wood solar drying device with latent heat storage system, where WPCMs were added with a multi-row pipe. The result showed that time of exothermic reactions gradually decreased with an increase in the multi-row pipe (5, 7, 9 and 11), however, the heat transfer

coefficient increased with the increase in the multi-row pipe. Their average values were 17.8, 22.1, 24.7,  $26.4W/(m^{2.\circ}C)$ , respectively. It was supposed that the other parameters except the number of pipelines were kept constant. When the number of pipelines was less than 5, the variation of heat transfer coefficient with temperature was very low. When the number of pipelines was more than 11, the storage of sensible heat was not neglected. On the other hand, it was supposed that the other parameters except the wind speed were kept constant. The heat transfer coefficient increased with the increase in the wind speed. When the wind speeds were 1.0, 1.5, and 2.0m/s respectively, ratio of exothermic reaction rates was 0.89:0.94:1.00 respectively.

# Utilization of wax phase change materials in the construction system

Zhou Dunbai<sup>7</sup> produced paraffins/montmorillonite nanometer sized phase change materials (NSPCMs) and studied on its utilization on the surface of the wall. NSPCMs were prepared by using unmodified montmorillonite with paraffins. Adding NSPCMs into concrete generated phase change construction materials. The temperature distribution in phase change construction materials was analyzed with ANSYS software. The fluctuation in room temperature decreased with adding NSPCMs and the living comfort was improved.

Table 2. The heat properties of n-paraff	fin wax
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Molecular	Molecular	Melting	Melting heat
formula	weight	point (°C)	$(J \cdot g^{-1})$
C16H34	226	16.7	236.81
C17H36	240	21.4	171.54
$C_{18}H_{38}$	254	28.2	242.67
C19H40	268	32.6	-
$C_{20}H_{42}$	282	36.6	246.86
$C_{21}H_{44}$	296	40.2	200.83
C22H46	310	44.0	251.04
C23H48	324	47.5	234.30
C24H50	338	50.6	248.95
C25H52	352	53.5	-
C <sub>26</sub> H <sub>54</sub>	366	56.3	255.22
C27H56	380	58.8	234.72

# Utilization of wax phase change materials in the air conditioning system

Wang Yue <sup>8</sup> used WPCMs to recover the residual heat from the air conditioning system. Cold water was heated through WPCMs. It not only reduced the domestic electricity consumption / prices but also decreased impact of the air conditioning system on the environment.

### Utilization of WPCMs in the textiles products

Wang Xiaopeng <sup>3</sup> introduced that outlast technology, firstly served for NASA, used WPCMs that absorbed, stored and released heat for living comfort. WPCMs was permanently stored and protected in a polymer shell. Their name called microencapsulated phase change materials Thermocules <sup>TM</sup>. This Thermocules <sup>TM</sup> was absorbed into fabrics and fibers that were able to adjust skin's microclimate. When the skin become hot, the heat was absorbed. When the skin become cool, the heat was released.

# CONCLUSION

Based on the above discussion and review, WPCMs are widely used in different areas such as the solar energy, construction, air conditioning systems and the textiles products, etc. It is an urgent need for *Chinese* scientists to study on the properties of WPCMs, e.g. wax density is increased and its coefficient of thermal conductivity does not change at the same time therefore its thermal capacity is also increased. Best WPCMs used in the different areas should also be produced. Nano-technologies combine with wax to produce a new nano scale sized phase change material should also be considered. Wax is added into macromolecular materials to generate multiple phase change materials.

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# A CRITICAL REVIEW OF CORROSION INHIBITION BY PHOSPHONIC ACIDS

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Keywords: Corrosion inhibition, metals, surface analysis, AFM, SEM, adsorption isotherm

Phosphonic acids are effective chelating agents. They bind strongly to metal ions. Phosphonic acids have also been widely used as corrosion inhibitors along with various metal ions as  $Zn^{2+}$ . The corrosion of various metals such as mild steel, copper, and aluminium have been prevented by phosphonic acids. Phosphonic acids exhibit good corrosion inhibition efficiency in acid medium, alkaline medium, and neutral medium. They can be used along with other inhibitors such as calcium gluconate, sodium molybdate, and trisodium citrate. Phosphonic acids show synergistic effects with these inhibitors. Various surface analysis techniques such as FTIR spectra, SEM, AFM, and EDAX have been used to analyze the nature of protective film formed on metal surface. Usually, the protective film consists of Fe<sup>2+</sup>-phosphonic acid complex along with Zn(OH)<sub>2</sub> if Zn<sup>2+</sup> is used. When phosphonic acids are used to prevent corrosion of iron, the adsorption process obeys Langmuir adsorption isotherm.

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# Introduction

Phosphonic acids have been widely used as corrosion inhibitors<sup>1-77</sup>. Phosphonates or phosphonic acids are organic compounds containing R-PO(OH)<sub>2</sub> or R-PO(OR)<sub>2</sub> groups. Phosphonates are effective chelating agents that bind tightly to di- and trivalent metal ions, preventing them from forming insoluble precipitates (scale) and suppressing their catalytic properties. They are stable under harsh conditions. Phosphonates have a very strong interaction with surfaces, which results in a significant removal in technical and natural systems. Due to this strong adsorption, little or no remobilization of metals is expected. The hydrophobicity increased as the length of the alkyl group attached to the phosphonate head increases. Phosphonic acids tended to make tiles stain resistant, even after cleaning. On copper and steel, the phosphonic acids only slightly increased hydrophobicity. On the glass, the phosphonates bonded fairly poorly to the surface and slightly increased hydrophobicity.

Overall, all substances coated with the phosphonate SAM showed increased hydrophobicity<sup>76</sup>. The adsorptive and inhibitive properties of some phosphonic acids have been studied in neutral aqueous solutions and to some extent in acidic solutions.

# **Structure of Phosphonic Acid**



# **Commonly Used Organic Phosphonic Acids in Corrosion Inhibition**

ATMP: amino trimethylene phosphonic acid.

<u>EDTMP</u>: ethylene diamine tetra methylene phosphonic acid.

<u>HEDP</u>: 1-hydroxy ethylidene-1, 1-diphosphonic acid.

<u>DTPMP</u>: diethylene triamine penta methylene phosphonic acid.

# **Occurrence in Nature**

The first naturally-occurring phosphonate, 2-aminoethylphosphonic acid was identified in 1959 in plants and many animals, where it is localized in membranes. Phosphonates are quite common among different organisms from prokaryotes to eubacteria and fungi, mollusks, insects, and others. They were first reported in natural soils by Newman and Tate (1980).

# **Properties and Uses**

Phosphonic acids have been used in metabolic regulation and in the development of potential drugs against several metabolic disorders. The great potential of these compounds in biological applications resulted in an intense effort

Ref	-	7	ε	4	S	6	٢	8	6	10	П	12	13
Findings	Mixed inhibitor, thermodynamic parameters, Langmuir adsorption isotherm.	Anodic inhibitor, protective film consists of $Fe^{24}$ -EPA complex.	The inhibition efficiency decreased slightly with temperature as well as hold time immersion under open circuit potential conditions. The adsorp- tion of PHOS on ordinary steel surface obeyed Langmuir's isotherm.	Presence of PPA and PPPA increases the inhibition efficiency by decreasing the corrosion rate, adsorption of compounds tested on the Armeo iron surface obeys to Langmuir adsorption isotherm.	The results of the experimental surface analyses were compared to molecular modeling studies of the proposed HEDP-Fe(III)/Zn(OH) <sub>2</sub> / HEDP-Zn(II) protective film. The increase in Zn(II) concentration leads to more compact and adherent film formation with decreased corrosion rates of carbon steel.	At higher concentrations (>50 ppm), HEDP was found to be aggressive towards mild steel, but a formulation consisting of 25 ppm HEDP and 200 ppm calcium gluconate was found to protect mild steel effectively.	Anodic reaction.	Mixed inhibitor, protective film consists of Fe <sup>2*</sup> -ATMP complex and $Zn(OH)_2$ .	Mixed inhibitor, Langmuir adsorption isotherm, and physisorption process. This inhibitor can be also used as biocide; it has antibacterial effect against both gram-positive and gram-negative bacteria. The antibacterial activity of ATMP is a result of a combined effect of the pH solution and the chemical nature of the used phosphonate molecule.	Anodic inhibitor. Surface film has been analysed.	Mixed inhibitor. Surface film is composed of iron oxides/hydroxides, zinc hydroxide, heteropolynuclear complex [Fe(III), Zn(II)-BPMG] and WO <sub>3</sub>	Mixed inhibitor, presence of oxides/hydroxides of iron(III), Zn(OH) <sub>2</sub> and [Zn(II)-PBTC-ascorbate] complex in the surface film.	Ternary inhibitor. Surface films have been analysed.
Method	Gravimetry, electrochemical measurements and surface morphological studies.	Weight loss method, polarization study, AC impedance, FTIR, and UV-visible reflectance.	Weight loss, polarization curves, and electrochemical impedance spectroscopy	Electrochemical studies, gravimetry, potentiodynamic polarization, weight loss method	Weight loss method, X-ray photoelectron spectroscopy (XPS)	Weight loss method, electrochemical polarization, and AC impedance measurements.	Weight loss method, energy-dispersion x-ray analysis (EDX), and electrochemical tests	Polarization study, AC impedance and FTIR spectroscopy	Weight loss, potentiodynamic polarization, and electrochemical impedance spectroscopy (EIS) techniques.	Potentiodynamic polarization study and FTIR spectroscopy.	Potentiodynamic polarization and impedance studies, X-ray photoelectron spectroscopy (XPS), FTIR, and scanning electron microscopy (SEM).	Potentiodynamic polarization studies, impedance studies, X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectrum.	Gravimetry, electrochemical impedance and potentiodyna-mic polarization studies, X- ray photoelectron and reflection absorption FTIR spectroscopy.
Additive	I	50 ppm of Zn <sup>2+</sup>	cetyltrimethyl- ammonium bro- mide (CTAB)	I	zinc(II) ions (14, 20, and 30 ppm)	calcium gluconate	1	5 ppm Zn <sup>2+</sup>	1	50 ppm of sodium tungsta- te and 10 ppm of $Zn^{2+}$	tungstate and zinc ions	ascorbate and Zn <sup>2+</sup>	$Zn^{2+}$
Inhibitor	nexamethylene diaminetetramethyl- phosphonic acid (HMDTMP)	250 ppm of ethylphosphonic acid (EPA)	$7.5 \cdot 10^{-5}$ to $1.10^{-3}$ phosphonate (PHOS)	piperidin-1-yl-phosphonic acid (PPA) and (4-phosphonopipera-zin- 1-yl)phospho-mic acid (PPPA)	50 ppm of HEDP	1-hydroxyethane-1,1-diphosphonic acid (HEDP)	Low phosphonic multipolymer	250 ppm of amino (trimethylenephos-phonic acid) (ATMP)	amino(trimethylene phosphonic acid) (ATMP)	250 ppm of ATMP	N,N-bis(phospho-nomethyl)glycine (BPMG)	2-phosphonobutane-1,2,4- tricarboxylic acid (PBTC)	ascorbic acid, 2-phos-phonobutane- 1,2,4-tri-carboxylic acid (PBTC), 1- hydroxy-ethane-1,1-diphosphonic acid (HEDP) and nitrilotris-(methy lenephosphonic acid) (NTMP)
Medium	U.5 M HCI	120 ppm of CI <sup>-</sup> and 73.58 ppm of sulphate ion	Simulated cooling water	3% NaCl	30 ppm of chloride	Tap water	Cooling water	Rain water collected from roof top	1 M HCI	1	Low chloride aqueous medium	Low chloride environment	Low chloride aqueous medium
Metal	Carbon steel	Carbon steel	Ordinary steel	Iron	Carbon steel	Mild steel	Brass	Carbon steel	Carbon steel	Carbon steel	Carbon steel	Carbon steel	Carbon steel
No.		5	ю	4	s.	.0	7.	×.		10.	11.	12.	13.

Table 1. The use of various phosphonic acids as corrosion inhibitors

Ref	14	15	16	17	18	19	20	21	22	23	24
Findings	Anodic inhibitor. Surface films formed on mild steel exhibit high charge transfer resistance and low double layer capacitance, which suggests that the film is non-porous and hence protective.	Mixed inhibitor. Protective film consists of Fe <sup>2+</sup> -DTPMP complex, Fe <sup>2+</sup> -SG complex, and Zn(OH) <sub>2</sub>	A simple oxide- hydroxide/diphosphonate model of the interface is proposed.	It shows that the addition of these molecules decreases the corrosion current density and the corrosion rate.	Synergistic effect exists between $Zn^{2+}$ and PPA. Surface film analysis showed that in the absence of $Zn^{2+}$ , the protective film consists of Fe <sup>2+</sup> . PPA complex formed on the anodic sites of the metal surface, whereas in the presence of $Zn^{2+}$ , the protective film consists of Fe <sup>2+</sup> -PPA complex and Zn(OH) <sub>2</sub> .	A mixture of PBTCA and PVP acts as a synergic inhibitor and found to increase the inhibition efficiency to $96.7\%$ .	Co-addition of 15 ppm HEDP and 15 ppm silicate has shown antagonistic behaviour. The surface homogeneity increases in case of inhibitors mixture application and this provides good protection to carbon steel against corrosion in soft water solution.	Addition of molybdate increases the inhibition efficiency of triazole in a synergistic manner. VATP as a better corrosion inhibitor for mild steel in aqueous solution. Additionally the formulation consisting of VATP, sodium molybdate, and CTAB offered good corrosion inhibition efficiency.	The corrosion resistance of the carbon steel coated by the epoxy resin containing ATMP-modified clay was higher than that obtained for the system containing non-treated clay. Local electrochemical measurements performed on scratched samples revealed the inhibitive role of ATMP at the carbon steel/coating interface.	Presence of EDTPO promotes the repairing of the passive film defects on the Al surface through a sealing process, which increases the stability of the oxide film layer. The film shows greater uniformity, lesser porosity, and remains stable for longer immersion periods as compared to those grown in the absence of EDTPO.	A combined inhibition effect was achieved by adding both ATMP and $Zn^{2+}$ along with Tween 80. The formulation functioned as a mixed-type inhibitor.
Method	AC impedance studies, potentiodynamic polarization studies, and reflection- absorption spectroscopy.	Weight loss method, polarization study, AC impedance spectrum, atomic force micrographs, and FTIR spectra.	EIS, XPS, and GD-OES data.	Potentiodynamic polarization study, FT-IR spectroscopy, scanning electron microscope, and energy dispersive X-ray analysis.	Weight loss method, surface analysis, potentiodynamic polarization study, and Fourier transform infrared (FTIR) spectrum.	Weight loss method, open circuit potential measurements, and potentiodynamic techniques.	Electrochemical impedance spectroscopy, scanning electron microscopy (SEM), Tafel polarization techniques.	Weight loss measurement, potentiodynamic polarization, AC impedance measurement, FTIR, XRD, and EDXA.	X-ray diffraction, electrochemical impedance spectroscopy (EIS) and local electrochemical impedance spectroscopy (LEIS).	Electrochemical and atomic force microscopy measurements.	Luminescence spectra, FTIR spectra, XRD, XPS, and scanning electron microscopic studies.
Additive	sodium tungstate, Zn <sup>2+</sup>	sodium gluconate, Zn <sup>2+</sup>			Zn <sup>2+</sup>	Polyvinylpyrro- lidone (PVP)	silicate	cetyltrimethyl armnonium bromide (CTAB), sodium molybdate			Zn <sup>2+</sup> and polyoxyethylene sorbitan monooleate (Tween 80)
Inhibitor	1-hydroxyethane-1,1-diphosphonic acid (HEDP)	Diethylenetriamine- pentamethylenephos-phonic acid (DTPMP)	1,5-diphosphono-pentane	thiomorpholin-4-yl-methyl- phosphonic acid (TMPA) and morpholin-4-methyl-phosphonic acid (MPA)	piperidin-1-yl-phosphonic acid (PPA)	2-phosphonobutane-1,2,4- tricarboxylic acid (PBTCA)	1-hydroxyethylidene-1,1- diphosphonic acid (HEDP)	3-vanillideneamino-1,2,4-triazole phospho-nate (VATP), 3-anisali- deneamino-1,2,4-triazole phosphonate (AATP) and 3-(p- nitrobenzyli-dene)amino-1,2,4- triazole phosphonate (PBATP)	montmorillonite clay (MMT) with aminotrimethylphos-phonic acid (ATMP)	Ethylenediaminetetra-phosphonic acid (EDTPO)	aminotrimethylidene- phosphonic acid (ATMP)
Medium	Well water	60 ppm of Cl <sup>-</sup>	Na <sub>2</sub> SO <sub>4</sub> Solution	Natural seawater	3% chloride solution	Cooling water	Soft water	Natural aqueous environment	Epoxy resin	ethyleneglyco 1-water mixtures	Ground water
Metal	Mild steel	Carbon steel	Polished zinc substrates	Carbon steel	Armco iron	Carbon steel	Carbon steel	Mild steel	Carbon steel	Aluminium	304 stainless steel
No.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23	24.

1	ke	25	26	27	28	29	30	31	32	33	34	35	36	37
:	Findings	The observed synergistic behaviour can be due to the incorporation of the silicate gel-like network through organic phosphorous bonds. The hydrodynamic condition of the electrolyte leads to enhancement of inhibition efficiency, which indicates that the corrosion inhibition was mass transfer controlled.	Adsorption of the inhibitors was found to follow the Langmuir's isotherm.	CT resistance has been found to increase with time due to passivation of steel by PANI-ATMP pigment, indicating the protective nature of the coating.	Mixed inhibitor. Protective film consists of $Fe^{2^+}$ -2-CIEPA complex and $Zn(OH)_2$ .	Ternary inhibitor system. The film consisted of [Fe(III)-HEIBMPA- molybdate] complex, [Fe(III), $Zn(II)$ -HEIBMPA-molybdate] complex, oxides and hydroxides of iron and $Zn(OH)_2$ .	Anodic inhibitor. The inhibition effects were due to the formation of a protective film of the multipolymer inhibitor on the metal surface.	Mixed inhibitor. Surface film also supported the formation of insoluble complexes of phosphomate and ascorbate with metal ions as well as presence of $Zn(OH)_2$ on the metal surface.	Surface coverage degrees indicate that the adsorption pattern is consistent with the Tomkin isotherm	The predominant corrosion control mechanism of the zine-HEDP mixture was on the anodic (metal dissolution) reaction. The effectiveness of the zine-HEDP mixtures can enhance inhibition by increasing the zinc content of the mixture.	The presence of biocide CTAB with inhibitor gives the inhibition efficiency of about 80% for mild steel. CPB with inhibitor shows higher interference between biocide and inhibitor, whereas CPB alone is found to act as an inhibitor for copper (73%), which shows that the inhibitor might interfere in the biocidal action on copper.	The amount of zinc in the surface layer increases with $Zn$ /HEDP molar ratio, which suggests the formation of either the 2:1 complex as well as $Zn(OH)_2$ or the zinc salt of the complex.	The film to the surface of the material by affecting the early stage of film formation.	A combination of inhibitive ions such as citrate, 2-CEPA, and zinc ions at 25 ppm gives 96 % inhibition efficiency and this corrosion inhibition is thus to the formation of a protective film
	Method	Electrochemical impedance, Tafel polarization measurements, scanning electron microscopy (SEM) and EDAX analysis.	Miero-wave technique, 1H-NMR, 13C- NMR, 31P-NMR, Tafel polarization curves, weight loss techniques, and Fourier transform infrared spectroscopy (FTIR).	FTIR, UV and X-ray fluorescence, XRD, salt spray, open circuit potential mesurement and EIS	Polarization study, AC impedance spectra, and FTIR spectra.	Potentiostatic polarization study, reflection- absorption FTIR spectrum, and X-ray photoelectron spectra.	Weight loss measurements, electrochemical tests, potentiodynamic polarization, and energy-dispersive X-ray analysis (EDX).	Potentiostatic polarization studies, AC impedance studies, X-ray photoelectron spectrum, and reflection-absorption FTIR spectrum.	Tafel extrapolation , linear polarization techniques and current-potential curves	1	Polarization studies.	Scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS).	Immersion test.	Weight-loss method and electrochemical impedance spectroscopy.
	Additive	sodium silicate		1	Zn <sup>2+</sup>	Zn <sup>2+</sup> and molybdate	I	Zn <sup>2+</sup> and ascorbate	,	Zinc	cetyltrimethyl ammonium and cetyl pyridinium bromide (CTA and CPB)		zinc sulfate	trisodium citrate and zinc ions
	Inhibitor	1-hydroxyethylidene 1,1- diphosphonic acid (HEDP)	<ol> <li>1-(4-methylpiperidine)</li> <li>methylenephosphonic acid (PO) and (1-(2<sup>2</sup>-aminoethylpiperidine))-</li> <li>2,2,4-tris(methylene-phosphonic acid) (3PO)</li> </ol>	polyaniline-amino trimethylenephosphonic acid (PANI-ATMP)	2-chloroethylphospho-ruic acid (2- CIEPA)	N-(2-hydroxyethyl) imino- bis(methylphos-phonic acid) [HEIBMPA]	Low phosphonic multipolymer	nitrilotnis(methylene-phosphonic acid) (NTMP)	o-methyl-, o-ethyl and o-isopropyl- p-methoxy-phenyldithiophosphonic acid ammonium salts (MTPA, ETPA and IPTPA, resp. )	1-hydroxyethylidene 1,1 diphosphonic acid (HEDP)	ATMP	HEDP	ATMP	2-carboxyethyl phosphonic acid (2-CEPA)
: ;	Medium	Soft water	3% NaCl	Epoxy resin	60 ppm of Cl <sup>-</sup>	Neutral aqueous environment	Synthetic water	Neutral aqueous environment	1 M HCI	Neutral oxygen- containing chloride solutions	Cooling water	Zinc ions	Industrial water	Chloride media
	Metal	Carbon steel pipelines	lron	Steel	Carbon steel	Carbon steel	Brass	Carbon steel	Steel	Carbon steel	Mild steel and copper	Mild steel	Carbon steel	Mild steel
	20.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.

Ref	38	39	40	41	42	43	4	45	46	47	48	49
Findings	The protective film consists of Fe <sup>24</sup> -SDS complex, Fe <sup>24</sup> -HEDP complex, and Zn(OH) <sub>2</sub> The HEDP-Zn <sup>24</sup> system shows good IE. The protective film consists of Fe <sup>24</sup> -HEDP complex and Zn(OH) <sub>2</sub> .	The protective film formed on the metal surface.	Low stability of calcium complexes compared with ferrous complexes, which facilitates the displacement of calcium ions from their complexes by ferrous ions forming soluble, unprotective, ferrous complexes. The zinc-HEDP mixtures give effective inhibition at zinc.	phenyltrimethoxysilane (PTMOS) based sol-gel film due to $\pi$ -interactions, the organic phosphonate adds to the protection efficiency of the sol-gel film	The mixture $(1.7 \text{x10}^{-5} \text{ M} \text{ HEDP} + 2.6 \text{x10}^{-3} \text{ M} \text{ SiO}_3)$ in the Ca <sup>24</sup> containing electrolyte is shown to be able to inhibit efficaciously the corrosion of iron at room temperature, considering uniform corrosion at pH 7 or pitting corrosion at pH 11.	Films consisted of hydrous ferric oxides (Fe(OH) <sub>3</sub> and FeOOH) with small amounts of Fe-NPMG complex, ZnO, and corrosion products.	VATP showed better protection over the other inhibitors used. The dissolution of copper in presence of VATP and AATP with biocide mixture is negligible compared to blank.	SATP was found to be the best corrosion inhibitor compared to the other compounds.	The formulation consisting of AATP, Mo and CTAB offered good corrosion inhibition efficiency	PPPA has a strongly inhibitive effect on chloride pitting corrosion. It seems that the addition of the NCH <sub>2</sub> PO <sub>3</sub> H group (center adsorption) in the PPA para-position, giving PPPA, reinforces the active sites of this molecule and consequently increases its inhibition efficiency.	Mixed-type inhibitors. Langmuir adsorption isotherm.	At the corrosion potential, electrochemical impedance diagrams exhibited a high frequency loop, which was ascribed to the charge transfer process, and a low frequency loop that was related to diffusion across the protective film. Analysis of impedance spectra in terms of an equivalent circuit model showed that the corrosion behaviour of the metal - layer solution system was strongly dependent on the physicochemical moneties of the corrosion products - corrosion inhibitor protous layer
Method	Weight loss method and FTIR spectroscopy.	Polarization, impedance measurements, FTIR, XRD, and EDXA	Electrochemical study.	Potentiodynamic polarization and electrochemical noise measurements, scanning electron microscopy (SEM) and Auger electron spectroscopy (AES).	Electrochemical measurements, mass-loss measurements, SEM, and EDX.	Polarization, electrochemical impedance measurements, XPS, and AES spectra.	Potentiodynamic polarization measurement and electrochemical impedance spectroscopy (EIS).	Weight loss, potentiodynamic polarization, and AC impedance methods.	Weight loss and electrochemical polarization techniques.	Electrochemical measurements and potentiodynamic polarization studies.	Weight loss and polarization measurements.	Electrochemical impedance measurements.
Additive	Zn <sup>2+</sup>	biocide	zine and calcium		sodium metasilicate pentahydrate Na <sub>2</sub> SiO <sub>3</sub> ·5H <sub>2</sub> O	Zn <sup>2+</sup>	1	1	Mo and CTAB	,	,	Zn <sup>2+</sup>
Inhibitor	sodium dodecyl sulphate (SDS) and 1-hydroxyethane-1,1-diphosphonic acid (HEDP)	3-benzylideneamino-, 3- cinnamalideneamino-, 3- salicylideneamino-, and 3-(p- nitro)benzyli-deneamino-1,2,4- triazole phosphonate (BATP, CATP, SATP and PBATP)	1-hydroxyethylidene-1,1- diphosphonic acid (HEDP)	phenylphosphonic acid (PPA)	HEDP	N -phosphonomethyl-glycine (NPMG)	3-varillideneamino and 3- artisalideneamino-1,2,4-triazole phosphonate (VATP and AATP)	3-salicylideneamino-1,2,4-triazole phosphonate (SATP)	3-benzylideneamino, 3-cinnamy- ledeneamino and 3-anisalideneami- 1,2,4-triazole phospho-nate (BATP, CATP and AATP, resp.)	piperidin-1-yl-phosphonic acid (PPA) and (4-phosphono-piperazin- 1-yl) phosphonic acid (PPA)	3-benzylideneamino-and 3-cinnamy- lidene-amino-1,2,4 triazole phos- phonate (BATP and CATP, resp.)	N-phosphonomethyl-glycine (NPMG)
Medium	Rain water	Neutral aqueous solution	Neutral oxygen- containing solutions	Organic anions	Industrial hard water containing 3·10 <sup>-3</sup> M Ca <sup>2+</sup> ions	Chloride solution	Neutral aqueous environment	Natural aqueous environment	Natural aqueous environment	NaCl	0.1 M HCl	Chloride solutions
Metal	Carbon steel	Copper	Mild steel	Sol-gel film on aluminium	Mild steel	Carbon steel	Copper	Mild steel	Mild steel	Iron	Mild steel	Carbon steel
No.	38.	39.	40.	41.	42.	43.	44.	45.	46.	47.	48.	.69

	Ref	50	51	52	53	54	55	56	57	58	59	60	61	62	63
	Findings	The protective film consists of $Fe^{2t}$ -HEDP complex and $Zn(OH)_2$ .	The corrosion inhibition efficiency of DTPMP+ $Zn^{2+}$ is found to be 80% after 24 hours.	Presence of $Zn^{2+}$ facilitates the transport of HEDP from the bulk of the solution to the metal surface; both the anodic reaction and cathodic reaction are controlled effectively.	Presence of more than 1,200 ppm of chloride ions, temperatures greater than or equal to 42.5°C, the combination of HEDP, molybdate and zinc could not control the corrosion in a cooling water system	Inhibition efficiency increases when the phosphomate- $Zn^{2+}$ complex remains in solution in soluble form. Inhibition efficiency decreased when the phosphomate- $Zn^{2+}$ complex was precipitated in the bulk of the solution.	A qualitative model is proposed, which entails passivation of the metal surface through anodization of the metal by PANI and formation of an insoluble iron-dopant salt at the metal surface.	Anodic inhibitor. The film was composed of an iron oxide/hydroxide mixture incorporating the organic compounds. The inhibitive molecules interact with the iron oxides.	Mixed inhibitor.	The nature of the film formed and the mechanistic aspects of film formation has been analysed.	The protective film consists of Fe^{2*-} HEDP complex, Fe^{2+}-CMC complex, and Zn(OH)_2.	Anionic complex can behave as an anodic, passivating inhibitor at a concentration as low as 0.00015M (20 ppm zinc-32 ppm HEDP mixture).	The adsorption of MMPA on aluminum oxide (Al <sub>2</sub> O <sub>3</sub> ) prevents the adsorption of chloride ions and leads to the formation of an insoluble aluminum complex of MMPA, which explains the significant decrease of the aluminum dissolution rate in the aggressive medium.	The protective film has been analysed.	The surface layer was composed of a mixture of slightly soluble $Za^{2+}/DPMG$ and zine hydroxide.
	Method	1	Voltammetric, gravimetric, and electrochemical methods.	UV-visible, FTIR, and huminescence spectroscopy.	1	Polarization and weight loss method.	Scanning reference electrode technology (SRET).	Steady-state current-voltage curve, electrochemical impedance measurement, and x-ray photoelectron spectros-copy (XPS).	Luminescence, XRD , FTIR spectra, pit morphology, and scanning electron microscopy.	Open circuit potential-time, polarization, impedance, and luminescence measurements.	Weight loss study, X-ray diffraction (XRD), FTIR and polarization study.	,	Direct current and alternating current techniques.	Weight change method, polarization, AC impedance technique, UV-VIS reflectance, UV-VIS luminescence, and ESCA techniques	Atomic force microscopy and X-ray photoelectron spectroscopy (XPS).
	Additive	Zn <sup>2+</sup>	Zn <sup>2+</sup>	Zn <sup>2+</sup>	sodium molybdate and zinc	Zn <sup>2+</sup>		1	50 ppm of Zn <sup>2+</sup>	Zn <sup>2+</sup>	cCarboxymethyl cellulose (CMC) and $Zn^{2+}$	Zn <sup>2+</sup>		zinc	bivalent cations ( $Ba^{2+}$ , $Sr^{2+}$ , ( $a^{2+}$ and $Zn^{2+}$ )
	Inhibitor	HEDP	DTPMP	sodium salt of HEDP	HEDP	phosphonates	sulfonic acid and phosphonic acid	fatty amines in association with phosphonocarboxylic acid	100 ppm of 3-phospho-nopropionic acid and 150 ppm of Tween 80 (polyoxyethylene sorbitan monooleate)	3- phosphonopropionic acid (3- PPA) and Triton X-100.	1-hydroxyethane-1,1-diphosphonic acid (HEDP)	HEDP	morpholinemethylene-phosphonic acid (MMPA)	tartrate with organophosphoric acid (2-carboxyethylphos-phonic acid)	N,N-di(phosphono-methyl)glycine (DPMG)
	Medium	Chloride solution	Neutral medium	60 ppm chloride	Chloride ions	60 ppm chloride ion	polyaniline (PANI)	200 ppm NaCl	Ground water	Ground water	60 ppm CI <sup>-</sup>	Oxygen containing aqueous solutions	Neutral aqueous chloride solutions	Oxygen saturated environment	1
	Metal	Carbon steel	Carbon steel	Mild steel	Carbon steel	Carbon steel	Mild steel	Carbon steel	304 stainless steel	304 stainless steel	Mild steel	Mild steel	Aluminum	Steel	Iron
	No	20.	51.	52.	53.	54.	55.	56.	57.	58.	59.	60.	61.	62.	63.
_	_					•			•			•	•		

Ret	64	65	90	67	89	69	70	71	72	73	74	75
Findings	The protective film is found to consist of $Fe^{24}$ -phosphonate complex and $Zn(OH)_{23}$ it is found to be luminescent.	Mixed inhibitor.	Mixed inhibitor.	The protective film consists of Fe <sup>2+</sup> -2-CI EPA complex and $Zn(OH)_2$ .	Langmuir adsorption isotherm.	Mixed inhibitor	Langmuir adsorption isotherm.	Mixed inhibitor. The protective film consisted of $Fe^{2n}$ -2-Cl EPA complex, Zn(OH <sub>2</sub> <sub>2</sub> and oxides of iron. The film was found to be semiconducting in nature.	The inhibition efficiency of aminophosphonic acids can be increased by halide ions. In the presence of aminophosphonic acids, the anomalous behavior of halide ions can be eliminated during anodic polarization.	Film showed the presence of the elements iron, phosphorus, nitrogen, oxygen, earbon, and zine. Deconvolution spectra of these elements in the surface film showed the presence of oxides/hydroxides of iron(III), Zn(OH) <sub>2</sub> , and [Zn(II)-BPMG] complex.	Langmuir's adsorption isotherm.	Langmuir's isotherm.
Method	Weight loss method, X-ray diffraction, Fourier transform infrared, and luminescence spectroscopy.	Weight loss method, polarization study, and X-rav diffraction.	Polarization study and fluorescence Spectra.	UV-luminescent.	Linear polarization and anodic polarization curve methods.	Weight-loss study, UV-visible reflectance spectroscopy and X-ray diffraction.	Polarization measurement, Fourier transform infra-red spectroscopy, and X-ray photoelectron spectroscopy	Weight-loss, electrochemical methods, polarization study, UV-visible absorption, and reflectance spectra.	Polarization measurement, scanning electron microscopy, FTIR reflection- absorption and X-ray photoelectron spectroscopy techniques.	Potentiodynamic polarization, X-ray photoelectron spectroscopic analysis (XPS), dDeconvolution and FTIR spectroscopy, SEM.	Weight loss measurement, potentiodynamic polarization, electrochemical impedance spectroscopy (EIS) techniques, and scanning electron microscope (SEM)	NMR and IR spectroscopy, Tafel polari- zation curve, weight loss technique, and FTIR spectroscopy
Additive	$Zn^{2+}$	Zn <sup>2+</sup>	Zn <sup>2+</sup>	Zn <sup>2+</sup>	,	Zn <sup>2+</sup>		Zn <sup>2+</sup>	halide ions	zinc		1
Inhibitor	Carboxymethylphos-phonic acid (CMPA) and 2-carboxyethyl- phosphonic acid (2-CEPA)	ethylphosphoric acid (EPA)	ethyl and 2-carboxy-ethyl phosphonic acid (EPA and 2-CEPA, resp.)	2-chloroethyl phospho-nic acid (2- Cl EPA)	N-sulfonated arnino-dimethylene- phosphonic acid (SADP) compared with HEDP (1-hydroxy-ethylidene- 1,1-diphos-phonic acid and annino- trimethylenephosphonic acid (ATMP)	HEDP	nitrilottis(methylene-phosphonic acid) (NTMP)	2-chloroethyl phosphonic acid (2-CI EPA)	aminophosphonic acids	N.N-bis(phosphono-methyl)glycine (BPMG)	hexamethylenediarnine tetra(methylene phosphonic acid) (HMDTMPA)	thiomorpholin-4-yl-methylphos- phonic acid (TMPA) and morpho- lin-4-yl-methyl-phosphonic acid (MPA)
Medium	60 ppm CI	60 ppm Cl <sup>−</sup>	60 ppm Cl <sup>-</sup>	60 ppm Cl <sup>-</sup>	H <sub>2</sub> SO <sub>4</sub> solutions	60 ppm Cl <sup>−</sup>	saturated Ca(OH) <sub>2</sub> solution	60 ppm Cl <sup>-</sup>	$H_2SO_4$	low chloride aqueous environment	1.0 M HCl solution	3% sodium chloride
Metal	Carbon steel	Mild steel	Mild steel	Carbon steel	0Crl3 stainless steel	Mild steel	Steel	Carbon steel	Armco iron	Carbon steel	Carbon steel	Iron
Z	64.	65.	66.	67.	68.	69.	70.	71.	72.	73.	74.	75.

Table 1 (cont.). The use of various phosphonic acids as corrosion inhibitors

directed to the development of efficient synthetic methods for their preparation, with particular attention to stereoselective synthesis. Phosphonic acids are also used as corrosion inhibitors in concrete, coatings, rubber blends, anti-freeze coolants, ship ballest compartments, preservation of organic products, coal slurries, etc<sup>77</sup>. Phosphonic acids are also good complexing agents. Among other phosphonic acids, diethylene triamine penta methylene phosphonic acids in particular can be used as scale and corrosion inhibitor in circulating cool water system and boiler water, and especially good in alkali circulating cool water system without additional pH regulation. It can also be used as scale and corrosion inhibitor in oilfield refill water, cool water and boiler water with high concentration of barium carbonate. It can also be used as peroxide stabilizer, chelating agent in weaving and dyeing industry, pigment dispersant, microelement's carrying agent in fertilizer and concrete modifier. In addition, DTPMPA is used in papermaking, electroplating, acid cleaning and cosmetics. Various phosphonic derivatives are widely used chemicals for corrosion inhibition in neutral aqueous solutions. Phosphonic acids find wide application in the corrosion protection of various metals in different environment.

# **Review and Discussion**

The use of phosphonic acids as corrosion inhibitors are discussed in the following section.

# Metals

Phosphonic acids have been used to control the corrosion of various metals such as mild steel<sup>1-6</sup>, brass<sup>7,30</sup>, copper<sup>34,39,44</sup>, aluminium<sup>23,61</sup>, stainless steel<sup>24,57,58,68</sup>, sol-gel film on aluminium<sup>41</sup>, Armco iron<sup>18,72</sup>, and zinc<sup>16</sup>.

### Medium

The inhibition efficiency of phosphonic acids in controlling corrosion of metals in various environments has been investigated. Acidic medium<sup>1,9,32,48,68,72,74</sup>, alkaline medium<sup>2,4,5,11-13,15,16,18</sup>, and neutral medium<sup>3,6-8,14,17,19-21</sup> have been used for this purpose.

# Inhibitor

Phosphonic acids have been used alone or in combination with other inhibitors such as Zn<sup>2+2,5,8,14,15,18</sup>, CTAB<sup>3,21,34,46</sup>, calcium gluconate<sup>6</sup>, tungstate<sup>11</sup>, ascorbate<sup>12</sup>, silicate<sup>20,25</sup>, sodium molybdate<sup>21,29,31,53</sup>, and trisodium citrate<sup>37</sup>.

# Methods

Various methods have been used to evaluate the inhibition efficiency of phosphonic acids. Weight-loss method<sup>2-7</sup>, Electrochemical studies (polarization and AC impedance)<sup>1-4</sup>. <sup>6-12</sup>, FTIR spectra<sup>2,8,10-13</sup>, UV-visible reflectance spectra<sup>2,14,24</sup>, XPS<sup>5,11-13</sup> and surface analysis by SEM<sup>11,20,24,25</sup>, AFM<sup>63</sup>, and EDX<sup>7,21,25,30</sup>.

# Findings

Using phosphonic acids as inhibitor, above studies have been conducted and following findings were reported:

Isotherm: Generally, the adsorption of phosphonic acids on metal surface obey Langmuir adsorption isotherm as supported by the studies of Laamari et al, Touir et al, and Amar et  $al^{1,3,4,26}$ ; and Temkin isotherm as supported by Karakus et  $al^{32}$ . Adsorption of phosphonic acids on metals like iron, carbon steel obey adsorption isotherm.

Type of inhibitor: Phosphonic acids have been used as mixed inhibitor  $^{1,\,8,\,9,\,11,\,12}$  and anodic inhibitor  $^{2,\,10,\,14,\,30}.$ 

Nature of protective film: The protective films formed on metal surface when phosphonic acids are used as corrosion inhibitors have been analyzed by FTIR, UV, SEM, AFM, and XPS. It is observed that the protective films consists of  $Fe^{2+}$  inhibitor complex is formed on anodic sites of the metal surface and Zn(OH)<sub>2</sub> formed on the cathodic sites of the metal surface<sup>2, 10, 12</sup>.

The use of various phosphonic acids as corrosion inhibitors are summarized in Table 1 above.

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# THE EFFECT OF DIFFERENT GROWING AREA ON THE Cu, Mn AND Zn CONTENT OF WINTER WHEAT

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# Keywords: Copper; Manganese; Zinc; Mass spectrometry; Environmental chemistry

In the present study the contents of Cu, Mn and Zn were investigated in the grains of winter wheat (*Triticum aestivum* L.). Plant samples were collected of the year 2004 when the weather conditions were very humid and wet. Samples were harvested and collected from five experimental stations of the Hungarian national long-term fertilization trials. These stations are as follows: Bicsérd, Iregszemcse, Karcag, Nagyhörcsök and Putnok. These experimental fields have different types of soil and climatic conditions. The elements content of samples were measured using inductively coupled plasma optical emission spectrometer (ICP-OES) and inductively coupled mass spectrometer (ICP-MS) followed by digestion with HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> solution. Data analysis was done using SPSS for Windows 13.0 Software package. All data were subjected to ANOVA method, but after detection of significant differences (P<0,05) data were subjected to Duncan's test to allow separation of means. The results of studies proved that the different regions caused significant (P<0.01) difference in the element content of winter wheat samples.

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# Introduction

In this study the Cu, Mn and Zn content of winter wheat grains from different growing fields were subjected to investigation. Microelement content of winter wheat grains harvested from different regions was found significantly different in the examination by Škrbić and Onjia.<sup>1</sup> These differences were due to different soil specifications like organic material content, pH, calm and mineral element content that could affect the availability of components.

Globally, wheat is cultivated in one of the three largest quantities of cereals. It is harvested 600 tonnes per year.<sup>2</sup> In Hungary production area of winter wheat varies from 1.0 to 1.2 million hectares.<sup>3</sup> Cereal crops are important source of mineral elements and nutrients for human beings.<sup>4</sup> In healthy human diet, 55% of the carbohydrates come from wheat.<sup>5</sup> Deficiency of mineral element intake is an alarming problem in the diet of human beings.<sup>6</sup>

Based on studies of the year 2000/01 National diet and nutrition survey of adults in the range of 19-64-year in United Kingdom reveals that, cereals and their products provide about 31% of the daily intake of Cu and 25% of Zn.<sup>7</sup> According to Szalay et al.<sup>8</sup> at least 70% of Mn intake comes from cereals in human diet. As per the literature data available so far manganese content of wheat grains is 32-88 mg kg<sup>-1</sup>, <sup>9,10</sup> or 25-40 mg kg<sup>-1</sup>, <sup>11,12</sup> and zinc concentration is 12-44 mg kg<sup>-1</sup>, <sup>9-12</sup> Copper content of cereal grains varies from 5 to 10 mg kg<sup>-1</sup>.

# Experimental

The Hungarian national long-term fertilization trials were set up in 1966 to study the effect of different NPK levels. The experiment marked as 18 has a split-split-plot design with 40 treatments in 4 replications. The crop rotation sequence was as follow: maize, maize, winter wheat and winter wheat. Samples of winter wheat variety Mv Csárdás were harvested and collected from Iregszemcse, Karcag, Nagyhörcsök and Putnok field stations. These experimental fields were having different soil types and climatic conditions. Tested samples are derived from the untreated control plots. Plant samples were collected from the year 2004. According to the Hungarian national weather service this year was also forecasted humid and wet.<sup>14</sup>

Bicsérd is located in Baranya Hills, in the South Transdanubian region of Hungary. The experimental field is 134 meters above the sealevel. It has humid climate. The soil is chernozem brown forest soil formed on loamy loess soils. It has slightly acidic pH. The humus layer is moderately thick and the topsoil is slightly leached. The cultivated layer has sandy loam mechanical composition. The soil has crumb structure with moderate soil moisture management. The cultivated layer has the following characteristics: pH (KCl): 5.45, CaCO<sub>3</sub>: 0 %, humus: 1.93%, S-Value: 17.4 mekv/100 g,\_whereof Ca<sup>2+</sup>: 79.4%; Mg<sup>2+</sup>: 17.6%; Na<sup>+</sup>: 0.1%; K<sup>+</sup>: 2.9%.

Iregszemcse is located in Transdanubian Hills, Outer-Somogy. The experimental area is 173 meters above the sea level. The soil is typical chernozem soil formed on slightly having clay loess sediments. It has slightly alkaline pH. The humus layer is moderately thick. The cultivated layer has sandy loam mechanical composition and the soil has crumb structure. Soil moisture is balanced. The cultivated layer has the following characteristics: pH (KCl): 7.49, CaCO<sub>3</sub>: 10.69%, humus: 2.69%, S-Value: 13.3 me/100 g,\_whereof Ca<sup>2+</sup>: 94.8%; Mg<sup>2+</sup>: 4.7%; Na<sup>+</sup>: 0.04%; K<sup>+</sup>: 0.63%.

Karcag is located in the central region of Tisza floodplain in the Great Hungarian Plain. The experimental area is 86 meters above the sea level. The soil is non-carbonated meadow chernozem soil formed on infusion loess. It has acidic pH. Organic matter content is moderate. The cultivated layer has clay loam mechanical composition and the soil has crumbly, grainy texture. Soil moisture is very favorable. The cultivated layer has the following characteristics: pH (KCl): 5.45, CaCO<sub>3</sub>: 0%, humus: 3.09%, S-Value: 20.4 me/100 g,\_whereof Ca<sup>2+</sup>: 76.1%; Mg<sup>2+</sup>:20.9%; Na<sup>+</sup>:0.3%; K<sup>+</sup>:2.7%.

Nagyhörcsök is located in Fejér county, Transdanubian region of Hungary. The experimental station is 140-150 meters above the sea level. It has arid climate. The soil is calcareous chernozem formed on loess. It has slightly alkaline pH. Humus layer is thick. The cultivated layer has loam mechanical composition and the soil has crumb structure with excellent soil moisture management. The cultivated layer has the following characteristics: pH (KCl): 7.3, CaCO<sub>3</sub>: 4.27%, humus: 3.45%, S-Value: 26.8 me/100 g, whereof Ca<sup>2+</sup>: 92.6%; Mg<sup>2+</sup>: 5.4%; Na<sup>+</sup>: 0.1%; K<sup>+</sup>: 1.9%.

Putnok is located in Heves-Borsodi Hills in North Hungarian Mountains. The experimental area is 163 meters above the sea level. The soil is non-podzolic forest infiltration clay soil. It has slightly acidic pH. The cultivated layer has clay loam mechanical composition. The soil has grainy, heavily clogged structure with heavy water retention and low drainage, permeability and available water resources. Natural nutrients service is poor. The cultivated layer has the following characteristics: pH (KCl): 5.00, CaCO<sub>3</sub>: 0%, humus: 2.29%, S-Value: 21.6 me/100 g, whereof Ca<sup>2+</sup>: 79.7%; Mg<sup>2+</sup>: 17.5%; Na<sup>+</sup>: 0.3%; K<sup>+</sup>: 2.5%.<sup>15</sup>

The elements content of samples were measured using inductively coupled plasma optical emission spectrometer (ICP-OES) and inductively coupled mass spectrometer (ICP-MS) following digestion with  $HNO_3$ - $H_2O_2$  solution. Data analysis was performed using SPSS for Windows 13.0 Software package. All data were subjected to ANOVA method, and when significant differences (P<0,05) were detected, Duncan's test was performed to allow separation of means.

# **Results and discussion**

The test results proved that different regions caused significant (P < 0.01) difference in the element content of winter wheat samples. Table 1 shows the Cu content of wheat samples from different field stations. Means (mg kg<sup>-1</sup>) express the means of four replications of the untreated control plots where samples were collected.

**Table 1.** Copper content of winter wheat (*Triticum aestivum* L.)

 grains from different regions

Production area	Means	SD
	(mg kg <sup>-1</sup> )	
Karcag	3,474	а
Bicsérd	4,025	b
Putnok	4,674	с
Nagyhörcsök	4,693	с
Iregszemcse	5,266	d

Samples, which were collected from Iregszemcse, have the highest Cu contents. Lowest Cu contents were measured in samples from Karcag. Our study reproduced the same results in these samples of wheat grains as the literature data, which is 3,6-7,6 mg kg<sup>-1.9,10</sup>

**Table 2.** Manganese content of winter wheat (*Triticum aestivum*L.) grains from different regions

Production area	Means	SD	
	(mg kg <sup>-1</sup> )		
Iregszemcse	13,323	а	
Bicsérd	14,505	а	
Karcag	29,989	b	
Nagyhörcsök	40,593	с	
Putnok	57,159	d	

During our investigation Mn content of samples from Putnok field stations found to be the highest one (Table 2). In the case of Iregszemcse and Bicsérd fields measured values are lower than literature data.<sup>9-12</sup>

**Table 3.** Zinc content of winter wheat (*Triticum aestivum* L.)grains from different regions

Production area	Means	SD	
	(mg kg <sup>-1</sup> )		
Karcag	14,283	а	
Iregszemcse	15,982	a, b	
Nagyhörcsök	17,521	b	
Bicsérd	25,023	с	
Putnok	45,225	d	

Table 3 shows the Zn contents of wheat grains from different field stations. The highest Zn content was measured in samples from Putnok and the lowest value was detected in grains from Karcag area. These results match with literature data.<sup>9-12</sup>

# Conclusion

During our investigations it was proved that different growing places of the Hungarian national long-term fertilization trials such as Bicsérd, Iregszemcse, Karcag, Nagyhörcsök and Putnok are having significant (P < 0.01) difference in the Cu, Mn and Zn content of winter wheat grains samples. These experimental stations have different soil and climatic conditions also.

In this study we compared our results with the work already done by other scientists. This investigation gives the same results in the case of Cu content as available in literature. During our investigation Mn content was found lower than the literature value in case of Iregszemcse and Bicsérd field stations. Literature data match with our results in the case of Zn contents.

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# EFFECTS OF SELENIUM AND SIMULTANEOUS EXPOSURE TO SELENIUM AND DIAZINON ON FEMORAL BONE STRUCTURE IN ADULT RATS

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Keywords: femoral bone, macroscopic analysis, histomorphometry, rat, selenium, diazinon

The present study aimed to investigate the macro- and the microscopic structure of femoral bone tissue in adult male rats after selenium (Se) and simultaneous exposure to Se and Diazinon (DZN). One month-old male, Wistar rats were randomly divided into three groups, of 10 males each. In the first group (EG1), rats were administered by Se at a dose of 5 mg of Na<sub>2</sub>SeO<sub>3</sub>/l in drinking water for 90 days. In the second group (EG2), animals received a drinking water containing 5 mg of Na<sub>2</sub>SeO<sub>3</sub>/l and 40 mg of DZN/l for the same time period. The third group of males without Se and DZN administration served as a control group (CG). Our results revealed a significant decrease in femoral length and cortical bone thickness in experimental groups (EG1, EG2) of rats compared to the control ones (CG). Rats from experimental groups (EG1, EG2) also displayed different microstructure in the middle part of the femur, where vascular canals expanded into central area of the bone while, in control rats, these occurred near endosteal surfaces only. Additionally, a smaller number of primary and secondary osteons was identified in experimental groups of these rats. A few resorption lacunae were observed in them simultaneously administered to Se and DZN. Based on these findings we can conclude that sub-chronic exposure to Se and simultaneous exposure to Se and DZN influences significantly macro- and microscopic structure of femoral bone tissue in adult male rats.

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# **1. Introduction**

Selenium is an essential trace element involved in several key metabolic activities, such as fertility, protection against oxidative damage, regulation of immunity and thyroid function.<sup>1,2</sup> In China, the deficiency of selenium in endemic regions causes Kashin-Beck disease (KBD) - a chronic endemic degenerative osteoarthritis.<sup>3-5</sup> The nutrition range between selenium essentiality and toxicity is fairly narrow.<sup>6</sup> Excess of Se causes abnormal bone and cartilage development and it is reported to be teratogenic.<sup>7</sup> Harr et al.<sup>8</sup> mention that 4 to 16 ppm Se in the diet causes adverse effects in the bones of rats; the bones were soft and the epiphyseal plates were easily separable. Chung et al.<sup>9</sup> found that apoptosis in mature osteoclasts supplemented for 6 hours in the additional presence of  $5\mu M$  or  $10\mu M$  sodium selenite was mediated by the mitochondrial pathway. Also excess of Se causes a death of osteoblast-like cells,<sup>10</sup> leads to abnormal bone and cartilage development.

Organophosphorus (OP) compounds are one of the most common types of organic pollutants found in the environment.<sup>11</sup> Residual amounts of OP pesticides have been detected in the soil, water bodies, vegetables, grains and other food products.12 Diazinon is an OP insecticide used to control a variety of insects.<sup>13</sup> Diazinon exerts its toxicity by binding its oxygen analog to the neuronal enzyme acetylcholinesterase (AChE), resulting in the accumulation of endogenous acetylcholine in nervous tissues and effects organs.<sup>14</sup> Recent evidence suggests that AChE may also have a functional role in bone.<sup>15-17</sup> Genever et al.<sup>16</sup> identified AChE expression in the bone cells osteoblasts. This fact suggest that AChE might have a function in a noncholinergic capacity of the bone, possibly through its ability to mediate cell function as demonstrated in various other tissues.<sup>18-20</sup> In order to support this hypothesis, binding motifs for osteogenic factors, including Cbfa-1, 17h-estradiol and vitamin D3, have been identified in the extended promoter region of the AChE gene.<sup>17</sup> In addition, OP pesticides as the potent inhibitors of AChE, cause a significant reduction in bone mass and density in individuals following chronic low-level exposure.<sup>15</sup> Several skeletal deformities such as an undulatin notochord and fused cervical rings induced by these pesticides have also been observed in the study by Misawa.21

To our knowledge, there are no studies describing the detailed histological analysis of the femoral bone after Se and DZN intoxication. Therefore, the main aim of the present study was to determine macro- and microscopic changes in the femoral bone of adult male rats exposed to Se and simultaneously exposed to Se and DZN in their drinking water diets.

Effects of Se and simultaneous exposure to Se and diazinon on femoral bone structure in adult rats

# **Experiments**

# Animals

Thirty 1-month-old male Wistar rats were obtained from the accredited experimental laboratory (number SK PC 50004) of the Slovak University of Agriculture in Nitra (Slovakia). These clinically healthy rats were randomly divided into three groups of 10 each. Male rats were used as they are less susceptible than female rats. The rats were housed individually in plastic cages in an environment maintained at 20-24°C,  $55\pm10\%$  humidity. They had access to water and food (feed mixture M3, Bonagro, Czech Republic) ad libitum.

The first experimental group (EG1; n=10 rats) was exposed daily to 5 mg/L Se, as Na<sub>2</sub>SeO<sub>3</sub>, in their drinking water for a total of 90 days. Ten 1-month-old males of the group EG2 received a drinking water containing 5 mg of Na<sub>2</sub>SeO<sub>3</sub>/1 and 40 mg of DZN/1 for the same time period of treatment. The third group (n=10 rats), without Se and DZN supplementation, served as the control group (CG). This study was approved by the Animal Experimental Committee of the Slovak Republic.

# Macroscopic analysis

At the end of the 90 days, all the rats were killed and their femurs were used for macroscopic and microscopic analyses. The femurs were weighed on an analytical scale to 2 decimal places and the length was measured with a sliding instrument.

# Microscopic analysis

For histomorphometric analysis, the right femurs were sectioned at the midshaft of the diaphysis and the segments fixed in HistoChoice fixative (Amresco, USA). The segments were then dehydrated in increasing grades (40 to 100%) of ethanol and embedded in Biodur epoxy resin (Günter von Hagens, Heidelberg, Germany) according to method described by Martiniaková et al.<sup>22</sup> Transverse thin sections (70-80  $\mu$ m) were prepared with a sawing microtome (Leitz 1600, Leica, Wetzlar, Germany) and fixed on to glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described.<sup>23</sup>

The qualitative histological characteristics of the compact bone tissue were determined according to the internationally accepted classification systems of Enlow and Brown<sup>24</sup> and Ricqlés et al.<sup>25</sup> Diaphyseal cortical bone thickness was measured by the Motic Images Plus 2.0 ML software. Twenty random areas were selected, and average thickness was calculated for each femur.

# Statistic

Statistical analysis was performed using SPSS 8.0 software. All data were expressed as mean  $\pm$  standard deviation (SD). The one-way analysis of variance (ANOVA), Tukey and Games-Howell tests were used for establishing statistical significance (significance level of P < 0.05) between experimental and control groups.

# **Results and discussion**

# Macroscopic analysis

Sub-chronic peroral exposure to Se and simultaneous exposure to Se and DZN, in drinking water for 90 days resulted in a significant decrease in femoral length and cortical bone thickness in these rats from both experimental groups (EG1, EG2) compared to the control group (CG). Femoral weight and cortical bone thickness were significantly increased in the Se-exposed rats (EG1) in comparison with those simultaneously exposed to Se and DZN (EG2). The results are summarized in Table 1.

**Table 1** Average femoral weight, femoral length and cortical bone thickness in the control (CG) and experimental (EG1, EG2) groups of rats.

Rat's	No.	Femoral	Femoral	Cortical bone
group		weight (g)	length (cm)	Thickness,mm
CG(1)	10	$1.05\pm0.17$	$3.94 \pm 0.09$	$0.572 \pm 0.054$
EG1(2)	10	$1.07\pm0.11$	$3.74 \pm 0.07$	0.528±0.051
EG2(3)	10	$0.93\pm0.07$	$3.75 \pm 0.07$	$0.507 \pm 0.050$
T-test		2:3	1:2; 1:3	1:2; 1:3, 2:3
		P<0.05	P<0.05	P<0.05

Data are expressed as means  $\pm$  SD. N – number of rats

Selenium is known to accumulate in the anterior pituitary of the pituitary gland, Thorlacius-Ussing et al.<sup>27</sup> mentioned decreased secretion of growth hormone (GH) and somatomedin C in rats after receiving 15 mg/L Na<sub>2</sub>SeO<sub>3</sub> in their drinking water, suggesting that growth retardation could be mediated by reduced GH and somatomedin C production. Ip<sup>26</sup> also reported a reduction in growth in rats following 12 weeks exposure to 5 mg/kg Na<sub>2</sub>SeO<sub>3</sub> in the diet. Gronbaek et al.<sup>28</sup> showed that Se treatment of 3.3 mg/L Na<sub>2</sub>SeO<sub>3</sub> in the drinking water for 35 days induced a significant reduction in circulating IGF-I of rats. These rats also disposed a significantly shorter tibia length. In the study by Misawa et al. (1982) diazinon-induced inhibition in growth of some skeletal elements, such as femur, tibia, metatarsi and digits of the leg in chick embryos were also demonstrated.

The thickness of cortical bone is an important parameter in the evaluation of cortical bone quality and strength. The value of cortical bone thickness in rats from the control group (CG) was different in comparison with values reported by Comelekoglu et al.<sup>29</sup> and Chovancová et al.<sup>30</sup> However; this discrepancy may be influenced by the different age of the rats in these studies. We could not compare the value of cortical bone thickness in rats from both experimental groups with published data, since it was not reported in previous studies.

Se either increases or decreases toxicity of various xenobiotics, including pesticides, depending on its amounts introduced into an organism.<sup>31</sup> According to many studies, Se is considered to be a relatively protective factor or antagonistic element against harmful impact of various toxicants.<sup>32-35</sup> In contrary, Se administration into the organism in higher amounts is able to induce oxidative stress and toxicity.<sup>36</sup> Moreover, Se is probably able to affect the atom of sulphur in the molecule of diazinon and amplificates the diazinon toxicity.<sup>37</sup> Therefore we suppose

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that significantly decreased femoral weight and cortical bone thickness could be mediated by higher oxidative stress and amplification of the DZN toxicity by the Se in rats from group EG2 compared to the EG1.

#### Microscopic analysis

Endosteal border of the femurs from the control rats (CG) was formed by non-vascular bone tissue in all views of the thin sections. The bone tissue contained cellular lamellae and osteocytes. Areas of primary vascular radial bone tissue were identified in anterior, posterior and lateral views. Vascular canals (branching or non-branching) radiating from the marrow cavity were also presented. Moreover, some primary and secondary osteons were especially found in the anterior and posterior views near the endosteal surfaces. In the middle part of the compact bone, a few primary and secondary osteons were identified. The periosteal border was again composed of non-vascular bone tissue, mainly in anterior and posterior views (Fig.1).



**Figure 1.** Microscopic structure of compact bone tissue in rat from the control group (1 - vascular canals radiating from marrow cavity, 2 - primary and secondary osteons).

The results of the qualitative histological analysis from the control rats corresponded with previous works.<sup>38-41</sup> We identified non-vascular and primary vascular radial and, irregular Haversian bone tissues. However, there was no evidence of true Haversian intracortical bone remodeling. It is generally known that aged rats and mice lack true Haversian cortical bone remodeling but not cancellous bone remodeling.<sup>40,42</sup> Therefore, some secondary osteons can be observed in the long bones (near the endosteal border). In our study, the newly formed remodeling units within compact bone originated from the endocortical surface and extended deep into the underlying compact bone. The same

findings have also been documented in the study of Reim et al.  $^{41}$  in 13 month-old male rats.

The rats from the both experimental groups (EG1, EG2) displayed a similar microarchitecture, except for the middle part of the femur in medial and lateral views. Vascular canals were shown to have expanded into the central area of the bone in these views. In some cases, the expansion was enormous and the canals occurred also near periosteal surfaces. A smaller number of primary and secondary osteons was also identified in these rats. Moreover, a few resorption lacunae were found near endosteal surfaces in rats simultaneously exposed to Se and DZN, which could indicate the early stage of osteoporosis (Fig.2).



**Figure 2.** Microscopic structure of compact bone tissue in rat from the experimental group (1 - enormous vascular canals radiating from marrow cavity, 2 - smaller number of primary and secondary osteons).

Prolonged intake of a high dose of Se and Se in combination with DZN induced changes in the middle part of compact bone in medial and lateral views where primary vascular radial bone tissue was present. We propose that the formation of this type of bone, in the central area of the femur, could be explained as an adaptive response to Se and DZN toxicity, to protect bone tissue against the cell death. It is generally known that Se at high doses induces apoptosis in mature osteoclasts9 and cell death in osteoblast-like cells.<sup>10</sup> In the study by Turan et al.<sup>43</sup> osteocyte loss was identified because of the destruction of the bone tissue and its replacement with large uncalcified volume of new bone matrix in rabbits fed excess Se (10 mg Na<sub>2</sub>SeO<sub>3</sub>/kg of diet for a period of 12 weeks). The authors also reported decreased biomechanical strength of the femur in Seexposed animals. Diazinon causes inhibition of AChE which is expressed by osteoblasts and is present along cement lines and, to a lesser extent, in osteoid. Therefore, AChE could have a role in the regulation of cell-matrix interactions and in the coupling of bone resorption to bone formation.<sup>15</sup> Therefore, higher bone resorption manifested by the presence of resorption lacunae in our study could be mediated by a low amount of AChE. In addition, Rangoonwala et al.<sup>44</sup> observed a progressive hypocalcemia in rats treated by sublethal dose of diazinon (daily intramuscular administration of 150 mg/kg b. w. and 225 mg/kg b.w. for 14 days). In is generally known that hypocalcemia inhibits calcitonin release. In the absence of the calcitonin, osteoclast activity is unregulated and bone resorption is accelerated<sup>45</sup> as it was documented also in our study.

In conclusion, sub-chronic exposure to Se and simultaneous exposure to Se and DZN in drinking water for 90 days significantly influenced macroscopic structure and also the microarchitecture of the femoral bone in adult male rats.

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Paper was presented at the 4th International Symposium on Trace Elements in the Food Chain, Friends or Foes, 15-17 November, 2012, Visegrád, Hungary

Keywords: herbal tea, black tea, green tea, fruit tea, aluminium.

Trace elements play an important role in human health. Some trace elements are essential, but some are harmful – especially in higher concentrations. Aluminum is not considered as an essential element for human beings, but has, in contrary, been correlated with various human pathologies for example Dementia, Parkinson and Alzheimer disease. Its uptake is mainly via food products for example tea, in the form of a food additive or via coming in contact with aluminium containing devices used during food processing and storage. The permissible daily dose for an adult is 60 mg of aluminium. Although no limit concentration for aluminium is given in the Codex Alimentarius Austriacus, the determination of the aluminium content in food and beverages is of great concern. In this study, different kinds of tea (black tea, green tea, fruit tea and herbal tea) were analyzed for their aluminium contents. The powders were brought into solution with a microwave assisted digestion unit at temperatures up to 220°C with diluted nitric acid as digestion medium. Furthermore, the aluminium concentrations have been determined in freshly prepared tea infusions in order to examine the extractable amount. The aluminium content in all solutions has been determined with ICP-OES (inductively coupled plasma-optical emission spectroscopy). Statistically significant differences between the different kinds of tea could be found, whereas the real tea types (green and black tea) contain more aluminium than fruit or herbal teas. The mean values of total concentrations of aluminium found were ( $0.91 \pm 0.31$ ) g/kg for green tea, ( $0.76 \pm 0.38$ ) g/kg for black tea, ( $0.23 \pm 0.09$ ) g/kg for herbal tea and ( $0.12 \pm 0.02$ ) mg/L for fruit tea. These results are in good agreement with literature data.

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# 1. Introduction

Tea, originating from China, has become a very popular beverage in the last 2000 years.<sup>1</sup> Positive effects on heath have already been reported in Chinese and Japanese documents long ago.<sup>2</sup> As tea contains various trace elements, the attention to the biological functioning of tea beverages has rose during the last few years. Tea infusions are prepared by extracting the tea leaves with hot water. Extraction times are about 5 minutes. Hot water dissolves proteins, vitamins, fibres or carbohydrates and furthermore some essential dietary metals which are mostly bound to polyphenols. <sup>3</sup> By consuming tea regularly, essential elements may therefore be taken in sufficient amount.<sup>4</sup>,<sup>5</sup>

Under acidic conditions as in presence of citric acid in tea with lemon – the absorption of elements, for example aluminium, may be increased.<sup>6</sup>

The total metal content in tea or tealeaves varies in different tea types (e.g. black or green) and also in these classes in certain ranges between different brands. Because of the relation of aluminium to adverse health effects when absorbed in excess, focus has put on this investigation. For black tea concentrations of around 900 mg/kg aluminium have been found, for green tea slightly higher average concentrations of 1,000 mg/kg. Leaves harvested for human consumption contain 300 to 1,500 mg/kg.<sup>7</sup> But more than 1,000 mg/kg is only rarely found in commercial tea leaves.<sup>12</sup>

Influencing factors on the total metal content of tealeaves are soil properties<sup>6</sup>, the age of the tealeaves and other environmental factors as for example rainfall or altitude of the growth area. Older tealeaves were reported to contain much more aluminium than young leaves <sup>8</sup> and the concentrations might even be as high as 2 to 3%.<sup>7</sup> Interestingly, it was also found that tea plants absorb aluminium from soils (for example Puerh tea plants) <sup>9</sup>, accumulate aluminium<sup>1</sup> and show even increased growth through aluminium uptake. <sup>10</sup> Chenery was the first who reported in 1955 the accumulation of unusual and high levels of aluminium (5 to 16 g/kg) by tea plants.<sup>8</sup>

As a consequence, the varying metal contents in the tealeaves also influence the metal content in tea infusions. An additional influencing factor hereby is the preparation method of the tea infusions.<sup>11</sup> Also the water composition for the hot tea infusions plays a role.<sup>21</sup> In general, the aluminium concentrations in tea infusions are found between 1 and 6 mg/L<sup>6</sup>, which was also found in other studies and in present investigation also. This makes tea infusions major sources for aluminium uptake.<sup>12, 13</sup>

Other ways of aluminium absorption in daily life are through food additives or via coming in contact with aluminium containing devices used during food processing and storage. On an average, a few milligrams of aluminium are the total dietary intake per day.<sup>12</sup>

Aluminium as trace element itself is not considered as essential element for human beings, but forms in contrast under certain conditions – for example acidic - potentially toxic species.<sup>14</sup> which can have harmful impacts on the human health. In the last decade aluminium has even been found to be responsible in Alzheimer's disease<sup>15</sup> Parkinson's disease and dialysis encephalopathy.<sup>16</sup>

Although no limit concentration for Aluminium is given in the Codex Alimentarius Austriacus, the determination of the aluminium content in food and beverages is because of its health risks at higher concentrations mentioned above, of great concern. In European countries the daily dietary exposure to aluminium was estimated on the average with 0.2 -1.2 mg/kg body weight per week; for highly exposed persons the value was as high as 2.3 mg/kg body weight per week.<sup>17</sup>

As tea counts to be an important source for aluminium uptake, different kinds of tea (black tea, green tea, fruit tea and herbal tea) were analyzed for their content of aluminium in this investigation. Total aluminium concentrations in tea powders from tealeaves as well as aluminium concentrations in freshly prepared tea infusions have been determined.

# Experimental

# **Tea samples**

Four different kinds of teas (black tea, green tea, fruit tea and herbal tea) have been taken for study under test. In these categories tea powders from various commercially available tea bags of different companies were used, as listed in Table 1. All samples were digested in duplicate. Samples without tea powders were also prepared as blank samples.

#### Table 1 Tea samples

Green tea	Black tea
G1	B1 (English Breakfast)
G2 with lemon	B2 (Irish cream)
G3	B3 (Earl grey)
G4	
G5	
G6	
G7	
G8 with lemon	
G9 w. lemongrass ingwer	
<i>n</i> =9	<i>n</i> =3
Herbal tea	Fruit tea
H1	F1 Pomegranate-Redcurrant
H2	F2 Strawberry
H3 (12 herbs)	F3 Strawberry Raspberry
	F4 Redcurrant-Cherry
	F5 Winter tea
<i>n</i> =3	n=5

# **Digestion of the tea samples**

All samples were prepared in the fume hood so that gas emissions from nitric acid were removed quickly. For the digestion approximately 0.150 g of tea powders were weighed into the microwave digestion flasks. 5 mL HNO<sub>3</sub> (65 ww%, p.a, Merck) and 4 mL distilled water was added. The flasks were then closed and the microwave assisted digestion started. The instrument used for the digestion process was a "StarT" from the company MLS. The temperature programme for the digestion process is listed in Table 2. After heating a cool-down phase followed for 20 minutes.

Table	2	Digestion	program
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Time [min]	Max. power [W]	Temperature [°C]
00:03:00	700	85
00:12:00	500	145
00:16:00	1000	180
00:21:00	1000	220
00:35:00	1000	220

After the samples cooled down to room temperature the flasks were opened and the completely dissolved tea powder solutions were filled up to 10 mL with distilled water.

# **Preparation of tea infusions**

One tea bag (approximately 1.5 - 2 g) and 100 mL of hot distilled water were used per tea infusion. The extraction time was 5 minutes.

Table 3 Tea sample masses [g] for digestion and preparation of the tea infusions

Green tea	<i>m</i> digestion	<i>m</i> tea powder in tea bags
	[mg]	for the infusions [g]
G1	108.2; 108.0	2
G2	103.1; 103.3	
G3	118.2; 124.3	
G4	106.6; 118.3	
G5	107.9; 108.8	
G6	106.9; 98.7	
G7	121.8; 106.7	1.75
G8	103.5; 113.6	1.75
G9	112.7; 102.2	1.75
B1	100.8; 105.2	
B2	110.4; 109.2	
B3	113.6; 97.7	2
H1	114.8; 127.7	2
H2	108.6; 98.0	2
H3	98.7; 112.7	2
F1	109.8; 103.3	
F2	106.3; 107.1	
F3	100.6; 107.1	
F4	106.3; 112.4	
F5	116.2; 106.7	3

### Determination of the aluminium concentration with ICP-OES

The aluminium content in all solutions (digestion solutions and tea infusions) has been determined with ICP-OES (inductively coupled plasma-optical emission spectroscopy) using an ICP spectrometer (Prodigy, Teledyne Leeman Labs, Hudson NH, USA) working in a simultaneous mode. The selected wavelength was  $\lambda = 396.152$  nm. For the preparation of standards the ICP Multielement Standard IV, Merck, Darmstadt, FRG was used. All standards were diluted with nitric acid (1 mol/L). This solution was also used as rinsing agent between the samples.

# **Results and discussion**

# Aluminium concentrations in the tea powders

The results for the total aluminium concentrations in the tea powders tested are shown in Table 4.

 Table 4 Total aluminium concentrations [g/kg] in the different tea

 powder samples

Туре	Sample	Content	(Mean content $\pm$ stan-
of tea		[g kg <sup>-1</sup> ]	dard deviation) [g kg <sup>-1</sup> ]
green	G1	$0.449\pm0.016$	
tea	G2	$0.716\pm0.009$	
	G3	$1.061\pm0.002$	
	G4	$1.250\pm0.151$	
	G5	$1.121\pm0.023$	$0.912\pm0.306$
	G6	$1.299\pm0.090$	
	G7	$0.997\pm0.052$	
	G8	$0.786\pm0.006$	
	G9	$0.534\pm0.016$	
black	B1	$0.385\pm0.005$	
tea	B2	$0.745\pm0.078$	$0.757 \pm 0.379$
	B3	$1.142\pm0.097$	
herbal	H1	$0.250\pm0.004$	
tea	H2	$0.128\pm0.037$	$0.226\pm0.089$
	H3	$0.301\pm0.029$	
fruit	F1	$0.181\pm0.040$	
tea	F2	$0.348\pm0.226$	
	F3	$0.217\pm0.010$	$0.216 \pm 0.077$
	F4	$0.181\pm0.096$	
	F5	$0.154\pm0.003$	

It can be noticed that within the four tea types different brands show wide ranges of aluminium concentrations that induces relatively high standard deviations. Nevertheless the results show significant visible differences between the mean concentrations of the different kinds of tea. This is illustrated in Figure 1.

From literature a relative high content of aluminium in black or green teas was already known. Higher contents of aluminium in the real tea types (green and black teas) were also determined in this study. Fruit or herbal teas were in this investigation found to contain lower aluminium concentrations (only around  $\frac{1}{3}$  or  $\frac{1}{4}$  of the concentrations in green or black teas).



Figure 1 Aluminium contents in the four different kinds of teas

#### Aluminium concentrations in the tea infusions

Tea infusions were prepared for selected samples. The results for the aluminium concentrations in the tea infusions are shown in Table 5.

Table	5	Aluminium	concentrations	[mg/L]	in	the	tea	infusions
from d	iff	erent kinds o	f tea					

Type of tea	Sample	Concentra- tion [mg/L]	Mean concentration ± standard deviation, [mg L <sup>-1</sup> ]
green	G1	4.34	
tea	G7	4.13	$4.22 \pm 0.25$
	G8	4.92	$4.55 \pm 0.55$
	G9	4.13	
black	B3	4.40	4 40
tea			
herbal	H1	0.10	
tea	H2	0.13	$0.12\pm0.02$
	H3	0.13	
fruit	F5	0.52	0.52
tea			0.52

The extractable amounts of aluminium are for black and green teas around 4.5 mg/L. Aluminium concentrations in tea infusions of fruit and herbal teas were found significantly lower than in the real tea types with 0.52 and 0.12 mg/kg in fruit and herbal tea, respectively.

Tea infusions have approximately 0.35% dry mass. Table 6 shows the extractable Aluminium concentrations for the four different tea types.

It can be noticed, that the amount of extractable aluminium is highest for green tea which brings this tea type - in despite of its otherwise very positive health effects because of its antioxidizing properties - in a negative context in terms of aluminium uptake through tea consumption and eventually possible adverse impacts on the human health. Black tea has the second highest extraction potential for aluminium and is therefore also very relevant in terms of possible health risks through uptake of aluminium. Aluminium is also quite extractable from tea powders for fruit tea infusions, but the average total content of aluminium in the tea powders in these two tea types is low Aluminium content of various tea powders and infusions

and so also the risk potential for aluminium uptake. Even less danger can be seen for herbal teas where only around 2% of the already low average total aluminium content is extracted.

Table 6 Aluminium concentrations [mg/L] in the tea infusions from different kinds of tea

Туре	Sample	Extractable Al	Mean [%] ± standard
of tea		[%]	deviation
green	G1	33.82	
tea	G7	14.49	24.22 + 9.17
	G8	21.94	$24.55 \pm 8.17$
	G9	27.06	
black	B3	13.48	12.40
tea			13.48
herbal	H1	1.37	
tea	H2	3.61	$2.16\pm1.25$
	H3	1.50	
fruit	F5	11.72	11.72
tea			11.72

# Comparison of the results with literature data

The determined concentrations ranges for the different tea types have been compared with literature data. In literature the focus for the determination of aluminium concentrations were mostly on green or black teas. This study extends the data also for fruit teas and herbal teas. Table 7 lists the data from this study and from literature.

 Table 7 Comparison of aluminium concentrations from this study with literature data

Literature	Aluminium concentration		
	[g kg <sup>-1</sup> ]		
	Green tea	Black tea	
STREET, A. et al. (2007) <sup>6</sup>	1.340	1.070	
Wrobel, K. et al. (2000) <sup>18</sup>	$0.919\pm0.03$	$0.759\pm0.03$	
Matsuura, H. et al. (2001) <sup>19</sup>		$0.807\pm0.06$	
Odegard et al. (1997) <sup>20</sup>		$0.90\ \pm 0.01$	
Mossion et al. (2008) <sup>21</sup>	0.4 - 1.0	0.4 - 1.0	
study	$0.912\pm0.31$	$0.757 \pm \! 0.38$	

It can be observed, that the results of this study confirm the findings for black and green tea with literature data. This is also true for the results obtained for the aluminium concentrations in the tea infusions that are shown in Table 8.

Table 9 shows the percentage of the extractable aluminium content from the tea powders to the tea infusions. Comparable results could be found for the extractable amounts of aluminium from black and green tealeaves.

It can be seen in Table 8 that the determined extractable amounts of aluminium vary in the different studies but that all results of green and black teas are almost in the same range and are a higher than the extractable amounts in fruit or herbal tea.

The results so found by the present underline the comparison with literature data and advance the knowledge of aluminium concentrations for fruit and herbal tea powders and their infusions.

 Table 8 Comparison of aluminium concentrations in tea infusions from this study with literature data

Literature	Aluminium concentration [mg L <sup>-1</sup> ] in tea infusions			
	Green tea	Black tea		
STREET, A. et al. (2007) <sup>6</sup>	3.2	2.3		
Matsuura, H. et al. (2001) <sup>19</sup>		$5.54\pm0.24$		
Flaten, T.P. (2002) <sup>12</sup>	1-6	1-6		
Mossion et al. (2008) <sup>21</sup>	0.06 - 3.24	0.06 - 3.24		
study	$4.33\pm0.35$	4.40		

 $\label{eq:comparison} \textbf{Table 9} \mbox{ Comparison of the extractable aluminium from this study} with literature data$ 

Literature	Extractable Aluminium concentration [% dry mass]		
	Green tea	Black tea	
STREET, A. et al. (2007) <sup>6</sup>	11.1	11.4	
Wrobel, K. et al. (2000) <sup>18</sup>	18 - 29	17 - 45	
Matsuura, H. et al. (2001) <sup>19</sup>		24.0	
study	$24.3\pm8.2$	13.5	

# Conclusions

The investigations of various tea types showed that significantly higher aluminium concentrations may be found in the real tea types (black and green), whereas the leaves of green tea contain the highest aluminium contents from all investigated types of tea which is about 0.9 g/kg. These results are in good agreement with aluminium concentrations in tealeaves reported in literature. The aluminium concentrations in black and green tea are three to four times higher than the investigated tea powder samples of fruit and herbal tea with around 0.2 g/kg in an average for both tea types. One reason for these findings is the significantly different organic matrix of real tea types and fruit or herbal teas. Also the real tea types are also known to be aluminium accumulators. Side by side with these results one may study the aluminium concentrations in the prepared tea infusions. Also here, significantly higher concentrations of aluminium could be found in green or black teas with 4.33 and 4.40 mg/L for green and black tea, respectively. As the aluminium uptake is reported with a few milligrams/day in literature, green and black teas can be a major source. On the other hand, fruit and herbal teas, only contain approximately 1/9 or even only 1/40 of the aluminium concentrations in tea leaves and therefore, the aluminium uptake from drinking tea is much lower than by drinking green or black tea.

The extractable amounts of aluminium found for green and black teas are also in the range reported in literature. Especially from green tea leaves that contain a higher total amount of aluminium compared to other tea types, the extraction potential is also the highest from the investigated tea types. From black tea leaves aluminium is also quite extractable. Fruit and herbal tealeaves contain only minor concentrations of aluminium and only small percentages are extractable so that these tea infusions do not count to major aluminium sources. Although tea (black or green) counts to be the biggest aluminium sources of human food products, the aluminium concentrations in the tea infusions is still rather low and should not pose threats to the human health.

Further studies will advance and develop the data based on the metal concentrations in tea powders and tea infusions.

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# EFFECTS OF CoCl<sub>2</sub> AND Co-EDTA ON TESTICULAR MORPHOLOGY AND SPERM COUNT IN MOUSE

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Keywords: Cobalt chloride, Cobalt-EDTA, in vivo model, mouse testis, sperm count

Cobalt (Co) is an essential trace element for mammals required for the synthesis of vitamin  $B_{12}$ . When cobalt was applied chronically it tends to accumulate in different organs and tissues that can induce pathological alterations. We focused our present study on the comparative effects of chronic exposure to cobalt chloride (CoCl<sub>2</sub>·6H<sub>2</sub>O) or cobalt EDTA (Co-EDTA) on testis and sperm count in mice of different ages. Both compounds were given in drinking water water with doses per day of 75 mg/ kg or 125 mg/ kg, respectively. Treated animals were sacrificed at different time intervals (on days 18, 25, 45 and 60). The most obvious changes in testis morphology - depletion and retardations in germ cell development, disorganization of seminiferous epithelium and SCO (Sertoli-cell-only) tubules were observed on the studies of 45 and 60 days respectively. In early puberty, testis weight was reduced in similar extend (with 25%) after high doses of both Co compounds whereas in early maturity and adulthood this parameter was reduced significantly and more severe after exposure to high dose CoCl<sub>2</sub> as compared to Co-EDTA, probably due to the stability of the complex Co-EDTA and its weaker absorption. Concerning gonado-somatic index and sperm count in mature animals we found similar tendency of more adverse effects with high dose of CoCl<sub>2</sub>. As a result of our work and data in literature we can conclude that the effects of chronic exposure to cobalt depend of the type of the compound used, dose, level, time duration as well as on the age of the animals under experiment. Cobalt could be considered as a possible risk factor for male fertility and health.

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# Introduction

Cobalt is naturally occurring, relatively rare element of the earth's crust<sup>1</sup>. Sources of exposure to cobalt and inorganic cobalt compounds are both natural and anthropogenic<sup>2</sup>. Cobalt is an essential oligo-element for mammals involved as a constituent of vitamin B<sub>12</sub> (cobalamin), mainly. Food and beverages represents the main source of cobalt for the general population<sup>3</sup>. By blood circulation cobalt could be delivered and subsequently accumulated in different organs like - liver, kidneys, hematopoietic organs, brain, reproductive organs, etc. Prolonged exposure to cobalt leads to different pathological alterations such as cardiomyopathy, impaired function of thyroid gland and liver. Cobalt has been shown to exert genotoxic and carcinogenic effects<sup>4</sup>. The crucial negative effects of cobalt on testis were rendered to its ability to induce conditions characterized with more or less decreased level of oxygen. Cobalt chloride is widely used pharmacological agent for inducing hypoxia. In the testis degenerative changes in adult seminiferous epithelium include vacuolation of Sertoli cells and germ cell nuclei reported by Elbetieha et al.5 In rats cobalt chloride (with <sup>58</sup>Co tracer) complexed with histidine, lysine, glycylglycine, EDTA, casein, or glycine was absorbed less then free cobalt chloride. Ethylenediamine tertraacetic acid (EDTA) is a widespread organic pollutant. It is used as anticoagulant for blood samples and decalcifying agent in histopathology, in non-alcoholic beverages. EDTA is powerful antioxidant and due to its ability to bind metals it is used in chelation therapy<sup>6</sup>.

Literature data show that oral exposure to cobalt compounds of adult animals causes reproductive and developmental alterations. We focused our study on the comparative effects of chronic exposure to CoCl<sub>2</sub> and Co-EDTA on testis morphology and sperm count in different ages of mice.

# Materials and methods

Pregnant ICR mice in late gestation were subjected to cobalt chloride (CoCl<sub>2</sub>·6H<sub>2</sub>O) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg kg<sup>-1</sup> or 125 mg kg<sup>-1</sup>, respectively, which continued until day 60 of the newborn pups. Cobalt compounds were applied via drinking tap water. Pure tap water was used as control. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in the institute's animal house at  $23 \pm 2$  °C and 12:12 h light-dark cycle in individual standard hard bottom polypropylene cages to insure that all experimental animals obtained the required dose of cobalt compounds.

Up to mid puberty (25 pnd) pups were influenced on  $CoCl_2$  or Co-EDTA at first via transplacental routes (during pregnancy) and subsequently via mothers' milk (during suckling period). On 25 pnd male mice were separated in personal cages, the doses were calculated and authenticated once a week according to their weight. Male pups were sacrificed on 18 pnd (early puberty), 25 pnd (mid puberty), 45 pnd (early maturity) and 60 pnd (maturity) respectively.

Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia and counted using Buerker's chamber. Data were statistically processed using Student's t-test. The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

# Results

# Histological evaluation of the testis

Histological sections of the testis were examined to determine whether the reduction in fertility of treated mice, reported in the literature, was in part due to a direct effect of cobalt on the structure of the testes. The most obvious changes in testis morphology we observed in early maturity and adulthood after treatment with high doses of both Co compounds. On day 45 spermatogenesis in the mouse is not completed but in control testis all the stages of spermatogentic cycle can be seen. Histological observation showed presence of mature spermatozoa and their release into the tubular lumen in stages VII-VIII of the cycle (Figure 1-A). On day 60 spermatogenesis is organized in 12 stages of classification by Clermont and Perey<sup>7</sup> and germ cells are arranged in 5-6 layers in seminiferous epithelium (Figure 2-A). After administration of high doses of CoCl<sub>2</sub> or Co-EDTA we found depletion of germ cells and retardation in germ cell development. Seminiferous epithelium was disorganized and undifferentiated germ cells were sloughed off in luminal area in many tubules (Figure 1-B, C; 2-B, C). SCO (Sertoli-cell-only) tubules were also seen (Figure 1-B; 2-C). In adult mice treated with high doses of both Co compounds we observed thinner seminiferous epithelium with less germ cells in the tubules. The diameter of seminiferous tubule was reduced probably due to impaired spermatogenesis.

# Quantitative data

Changes in the testes weight (TW) are indicative for altered spermatogenesis. In early puberty (d18) we found more severe effect of low dose of CoCl2 compared to Co-EDTA that was significant whereas high doses of both compounds induced similar reduction of 25% compared to control TW (Figure 3). On day 45 and day 60 after administration of 75 mg/kg of CoCl2 and Co-EDTA, TW remained in normal range and no difference was found between both groups. In contrast to puberty, during maturity and in adulthood CoCl<sub>2</sub>-125 mg kg<sup>-1</sup> induced significantly more damaging alterations in TW (respectively 35% and 30% lower than control on days 45 and 60) compared to Co-EDTA (15% reduction than control value on day 60, that is not significant). Simultaneously, gonadosomatic index (ratio TW to body weight) in late ages was also considerably more sensitive to CoCl<sub>2</sub> as compared to Co-EDTA (data not shown).

Evaluation of sperm count accumulated and isolated from vas deferens in maturity and adulthood demonstrated a wide deviation in this parameter in all of the experimental groups. Low doses of both Co compounds induced similar changes of sperm count on day 45, whereas high dose of CoCl<sub>2</sub> lead to 70% reduction of the investigated parameter compared to 35% decrease after Co-EDTA (Figure 4). Difference between the effects of high doses of both compounds was significant on day 60.



(A) Control



(B)  $CoCl_2 - 125 \text{ mg kg}^{-1} d^{-1}$ 



(C) Co-EDTA – 125 mg  $kg^{-1} day^{-1}$ 

**Figure 1**. Morphology of the seminiferous tubules on testis cross sections of 45-day old mice. HE, x 400. Depletion of germ cells and retardations in germ cell development after treatment with high doses of CoCl<sub>2</sub> or Co-EDTA (125 mg kg<sup>-1</sup> d<sup>-1</sup>) was observed.

# Discussion

The experimental results showed that treatment with CoCl<sub>2</sub> and Co-EDTA induced a lot of abnormalities in testis morphology that could later affect male fertility. Testicular histology of mature and adult animals was not different in mice treated by both Co compounds.



# (A) Control



(B)  $CoCl_2 - 125 \text{ mg kg}^{-1} \text{ day}^{-1}$ 



(C) Co-EDTA – 125 mg  $kg^{-1} day^{-1}$ 

**Figure 2.** Morphology of the seminiferous tubules on testis cross sections of 60-day old mice. HE, x 400. Depletion of germ cells and disorganization of seminiferous epithelium after treatment with high doses of CoCl<sub>2</sub> or Co-EDTA (125 mg kg<sup>-1</sup> day<sup>-1</sup>) was observed. Presence of SCO (Sertoli-cell-only) areas.



**Figure 3.** Changes in testis weight (TW) in different stages of development in mouse after treatment with CoCl<sub>2</sub> or Co-EDTA at two daily doses of 75 mg kg<sup>-1</sup> or 125 mg kg<sup>-1</sup>.



**Figure 4.** Changes in spermatozoa count in early and late maturity (day 45 and 60) in mouse after treatment with CoCl<sub>2</sub> or Co-EDTA at two daily doses of 75 mg kg<sup>-1</sup> or 125 mg kg<sup>-1</sup>.

After chronic exposure to cobalt Bitner et al.8 and Elbetieha et al.<sup>5</sup> reported structural changes in the testis including necrosis and degeneration of seminiferous epithelium. Our data support the findings of Lukac et al.<sup>9</sup> about sloughing of germ and Sertoli cells and shrinkage of the seminiferous tubules. Formation of empty spaces within the epithelium was also described (SCO areas). In the literature, morphometric analysis was reported to reveal significant decrease in relative volume of seminiferous epithelium in cobalt treated animals, whereas the relative volume of interstitium was significantly increased. Larger interstitium is probably due to increased Leydig cell (LC) volume that could predict elevated testosterone (T) levels. Pedigio et al.<sup>10</sup> presumed that cobalt interferes with local regulatory mechanisms in testosterone synthesis. Experimental treatment with cobalt influenced LC steroidogenesis - serum T-levels were significantly increased, while FSH and LH serum levels remained normal. Besides possible indirect effect on spermatogenesis cobalt readily crosses the blood-testis barrier. Subsequently, a direct cytotoxic effect of cobalt on spermatogenic and Sertoli cells is possible<sup>11</sup>. Mollenhauer et al.<sup>12</sup> suggested that testicular degeneration was not a primary response to cobalt but the testes become hypoxic due to both the blockage of veins and arteries by red blood cells and to the changes in permeability caused by thickening of basal lamina.

In early puberty (d18) we observed similar effect of high doses of  $CoCl_2$  and Co-EDTA on testis weight whereas in maturity and adulthood  $CoCl_2$  affects more severe this

parameter compared to Co-EDTA. Our finding is probably due to the stability of the complex Co-EDTA and its weaker absorption. Bitner et al.<sup>8</sup> and Elbetieha et al.<sup>5</sup> reported decreased weight of testes and epididymides while weight of seminal vesicles and preputial glands was significantly increased. It has been noted that the size and activity of secondary sex glands is clearly influenced by a variety of steroid hormones. Duration of cobalt exposure is very important for the subsequently induced abnormalities and for the following period of recovery<sup>4</sup>. Probably, the observed effects in adult animals are due to accumulated Co in the organism and its effect on testis morphology and TW.

Our data for reduced sperm count were in good agreement with previous studies of the rodent testis after chronic Co intoxication<sup>10</sup>. Impairment in this parameter was dosedependent and it increased as the concentration of cobalt increased. More pronounced were the changes in sperm count after high dose of CoCl<sub>2</sub>. These findings suggest that the effects of cobalt (II) depend on the type of compound used and on stability of its complex. Time duration and age of the experimental animals are also important.

In conclusion, our data and findings indicate that exposure to cobalt during perinatal and postnatal period affected testis morphology in mature and adult animals; reduced TW in pubertal and mature animals and decreased epididymal sperm count. The effects of CoCl<sub>2</sub> were more pronounced as compared to Co-EDTA induced alterations.

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# RESTORATION OF CONTAMINATED SOIL SITES WITH THE AID OF FAST GROWING TREES

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### Keywords: cadmium, lead, phytoremediation, poplar, soil, willow

Soil is basic means of agricultural production and subsequently the source of food for human population. Soil degradation and contamination is concerned of an issue worldwide. Several remediation techniques have been developed and applied to restore contaminated land and to keep soil properties on the sufficient level for the food production. Success of designed procedure is affected by metal presence and level of contamination. In experiments to extract metals from moderately and heavily metal-contaminated sites were investigated along with procedure to study the influence of organic fertilizers application on biomass production and a heavy metal uptake as well. The ability to accumulate risk elements in plant tissues has been proved, cadmium showed the best performance. Furthermore, a significant influence of biomass production on the total uptake of risk elements was proved. Organic fertilization had an adverse effect on the metal uptake. Extremely contaminated soils cannot directly be remediated by plants itself; they mainly need sufficient chemostabilization treatment before planting of trees.

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# Introduction

Soil contamination with heavy metals is a major environmental problem nowadays and affect not only the soil quality but also several other environmental parameters.<sup>1</sup> Restoration of contaminated soils using conventional technologies requires considerable economic resources. For this reason, an interest has developed to have decontamination cost effective techniques. Phytoremediation is one of them. This method provides acceptable economic and environmental aspects for cleaning up contaminated soils.<sup>2</sup> Phytoremediation is methods using plants for restoration of main functions of contaminated soils and is established on several principles: stabilization methods like phytostabilization and phytoimmobilization or removal methods as phytoextraction, phytodegradation and phytovolatilization.<sup>3</sup> Significant role in development of remediation methods plays selection of appropriate plant species for phytoremediation. <sup>4</sup> Adriano (2001) <sup>5</sup> categorized plants into several groups according to their ability to accumulate risk elements: metal excluders, indicators and accumulators (metallophytes). Metallophytes are plant species that able to grow in contaminated areas and accumulate heavy metals in aboveground biomass. Nevertheless, these plants are able to produce less biomass than other species. <sup>6</sup> Therefore, appropriate group of plants for phytoremediation techniques are fast growing trees that accumulate relatively lower amounts of heavy metals but provide a high biomass production. Generally higher

removal of risk elements by fast growing trees and utilization of their biomass production provide economic return of contaminated land.<sup>7</sup> According to pot experiments of Vysloužilová et al.<sup>8</sup> willows planted in two soils differing in level of contamination confirm their ability to accumulate Zn and Cd in plant tissues. All tested willow clones planted in highly contaminated Fluvisol from the alluvium of the Litavka River suffer from chlorosis, partial leaf fall and reduction in the yield of aboveground biomass. Nevertheless, willow clones are able to provide high biomass production and accumulate substantial amounts of Cd in aboveground biomass in medium contaminated Cambisol.

The aims of this study were to evaluate the effects of two phytoremediation techniques: 1) phytoextraction in medium contaminated soil and 2) chemophytostabilization in highly contaminated soil.

First, we investigated ability of selected clones of willows and poplars to accumulate Cd and Pb in their branches grown on moderately contaminated soil in sewage sludge and control treatments in field conditions and second, the effect of alkaline and phosphorus additives to stabilize Cd and Pb in heavily contaminated soil and observe changes in yield and in Cd and Pb content in willow in model pot conditions.

# **Materials and Methods**

# Soil samples.

Two soils differing in level of contamination and soil type were used in our study. Both contaminated soils are located near the Příbram town and this region belongs to the most polluted areas in the Czech Republic. <sup>9</sup> 1) Weakly acidic soil "Podlesí" with medium contamination was tested in the field experiment and was characterized as follows: modal Cambisol, CEC 166 mmol kg<sup>-1</sup>, C<sub>org</sub> 4.1 %, 8.3 mg Cd kg<sup>-1</sup>, 218 mg Zn kg<sup>-1</sup>, 1214 mg Pb kg<sup>-1</sup>. At the study site, the mean annual precipitations are 700 mm and mean annual temperature is 6.5 °C (Příbram meteorological station). 2) Slightly acidic soil "Litavka", highly contaminated, was used in the pot experiment and was characterized as follows: Fluvisol, CEC 55 mmol kg<sup>-1</sup>, C<sub>org</sub> 3.6 %, 53.8 mg Cd kg<sup>-1</sup>, 6172 mg Zn kg<sup>-1</sup>, 3305 mg Pb kg<sup>-1</sup>.

# **Field experiment**

The field experiment in "Podlesí" was started in year 2008. The each treatment (control; sewage sludge, 7.5 kg m<sup>-</sup> soil) used in the experiment was established in four replications. The plot of the each treatment was  $7.5 \text{ m} \times 3.9$ m. The experimental plots were planted with cuttings of fast growing trees from family Salicaceae (genus Populus poplars and genus Salix - willows). Two clones of willows (S. schwerinii × S. viminalis – VB1; S. smithiana – VB2) and two clones of poplars (P. nigra × P. maximowiczii -TP1; P. nigra – TP2) were used in each treatment. Planting distance between each 20 cm long cutting was  $1.3 \text{ m} \times 0.25$ m. After four years of planting in February 2012, branches (8 plant samples from each clone for each treatment) were harvested, measured, checked for fresh and dry biomass (dried at 60°C), ground and decomposed by dry-ash procedure. <sup>10</sup> Total Cd and Pb contents were determined by inductively coupled plasma with optical emission spectroscopy (ICP-OES) (Varian VistaPro, Varian, Australia).

### Pot experiment

In pot experiment, two different groups of soil additives (alkaline additives, phosphorus additives) were used as the metal stabilization agents in highly contaminated soil "Litavka". From alkaline additives were chosen highly soluble quick lime and poor soluble dolomite, from phosphorus additives fast P release superphosphate and slow P release rock phosphate. At the beginning of the experiment in 2010, air-dry soil was treated with 0.3 g N, 0.16 g P, and 0.4 g K per kg of the soil. All soil additives (quick lime – 7.3 g kg<sup>-1</sup> soil, dolomite – 21.6 g kg<sup>-1</sup> soil, superphosphate – 4.2 g kg<sup>-1</sup> soil and rock phosphate – 2.9 g kg<sup>-1</sup> soil) corresponding to individual treatments were thoroughly mixed with the experimental soil and than one 20 cm long cutting of willow (*S. smithiana* – VB2) was planted in each pot (containing 5 kg soil).

Four replications were used for each treatment (control, quick lime, dolomite, superphosphate, and rock phosphate). The pots were kept in an outdoor weather-controlled vegetation hall. After five months of plantation leaves and twigs were harvested, checked for fresh and dry biomass, ground and decomposed by dry procedure as well as soil samples were collected and analyzed. Soil samples were extracted with 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> (1:10 w/v) for 6 hours. <sup>11</sup> Plant-available Cd and Pb concentrations in soil extracts and total plant Cd and Pb contents were determined by ICP-OES.

# Data analyses.

The total element contents in plants, dry biomass and plant-available element concentrations in soil from individual treatments in field or pot experiments were evaluated by ANOVA (STATISTICA 9.0 software).

# Results

# **Field experiment**

Tested clones of fast growing trees planted in medium contaminated soil "Podlesi" showed high potential to accumulate Cd (Fig. 1). Both willow clones significantly accumulated more Cd compared to poplar clones. The highest amount of Cd accumulated willow clone VB1 in control treatment (54.2 mg kg<sup>-1</sup>) and the lowest amount of Cd poplar clone TP1 (16.3 mg kg<sup>-1</sup>). All tested clones in sewage sludge treatment accumulated less Cd compared to control treatment.



Figure 1. Cd content in branches of willow and poplar clones planted in medium contaminated soil treated with different treatments (C – control, SS – sewage sludge). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each clone.

Low mobile Pb was accumulated in branches of fast growing trees less compared to Cd (Fig. 2). Poplar clones accumulated higher Pb contents than Cd ones. The highest amount of Pb accumulated poplar clone TP1 in control treatment (34.4mg kg<sup>-1</sup>). The lowest amount of Pb accumulated poplar clone TP2 in sewage sludge treatment (16.8 mg kg<sup>-1</sup>). Poplar clone TP2 was in Pb accumulation ability similar as willow clones. As in the case of Cd, fast growing trees planted in control treatment accumulated more Pb compared to sewage sludge treatment.

Biomass production is one of the significant parameters for evaluating ability of plants for phytoextraction. Decreasing trend in biomass production was in order TP1>VB2>TP2>VB1, see Fig. 3. Nevertheless, a decrease in biomass production was significantly different only between poplar clones TP1 and TP2.



Figure 2. Pb content in branches of willow and poplar clones planted in medium contaminated soil treated with different treatments (C – control, SS – sewage sludge). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each clone.



Figure 3. Dry biomass of willow and poplar clones planted in medium contaminated soil treated with different treatments (C – control, SS – sewage sludge. Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each clone.

Biomass production of fast growing trees was higher in control treatment compared to sewage sludge treatment. It is interesting because it was conversely to previous years. It can be explained by several effects: 1) deficiency of nutrients for growth of fast growing trees in sewage sludge treatment after four years of start of field experiment, 2) competition with overgrown weeds, especially docks and 3) partial mortality of fast growing trees in sewage sludge treatment. In field conditions is very difficult to identify specific effects.

Total removal of risk elements plays the most important role in phytoremediation and is calculated by multiplication of plant element content and dry aboveground biomass. Figure 4 represents removal of Cd and figure 5 removal of Pb by plants. From both graphs, is obvious effect of biomass production on removal of both elements.



Figure 4. Removal of Cd by selected willow and poplar clones planted in medium contaminated soil treated with different treatments (C – control, SS – sewage sludge). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each clone.

Total removal of Cd was the highest by willow clone VB2, although the highest Cd content in branches was in willow clone VB1. The order of decreasing removal of Cd followed the order: VB2>TP1>VB1>TP2 with significantly different between clones VB2 and TP2.

Pb content in branches decreased in the order: TP1>VB1>VB2>TP2. Nevertheless, removal of Pb decreased in order: TP1>VB2>TP2>VB1. We can conclude that removal of Pb has similar trend as biomass production.



Figure 5. Removal of Pb by selected willow and poplar clones planted in medium contaminated soil treated with different treatments (C – control, SS – sewage sludge). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each clone.

Fast growing trees planted in sewage sludge treatment accumulated less Cd and Pb compared to control treatments. It can be related with low biomass production and low content of both elements in aboveground biomass which can be probably affected by higher sorption surface caused by organic matter derived by sewage sludge. Nevertheless, differences among treatments were not significantly different.

#### Pot experiment

Addition of both alkaline additives (quick lime, dolomite) significantly reduced plant-available Cd concentrations compared to the control (see Table 1). In the case of phosphorus additives, Cd immobilization was established only in the rock phosphate treatment. Slight increase in plant-available Pb concentrations after all soil additives applications was not significantly different.

**Table 1.** Mean plant-available Cd and Pb concentrations (±standard error of the mean; SE) in highly contaminated soil treated with different treatments (control, quick lime, dolomite, superphosphate, and rock phosphate) after five months of planting willow clone VB2. Using Tukey post-hoc test, treatments with the same letter are not significantly different.

Treatment	Element (mg kg <sup>-1</sup> )		
	Cd	Pb	
control	$3.11 \pm 0.09^{a}$	$0.14{\pm}0.04^{a}$	
quick lime	$0.27 \pm 0.04^{b}$	$0.26{\pm}0.05^{a}$	
dolomite	2.36±0.06°	$0.23{\pm}0.08^{a}$	
superphosphate	$3.51 \pm 0.07^{d}$	$0.19{\pm}0.05^{a}$	
rock phosphate	2.75±0.10 <sup>e</sup>	$0.24{\pm}0.10^{a}$	

Quick lime, dolomite and superphosphate applications increased Cd content in leaves compared to the control but not significantly (see Fig. 6). In contrast, rock phosphate decreased Cd content in leaves. From comparison of plantavailable Cd concentrations in soil, we can conclude that there is an indirect correlation after alkaline additives application and direct correlation after phosphate additives application. Otherwise, there was no effect of tested additives on decrease Cd content in twigs compared to the control.



Figure 6. Cd content in leaves and twigs of willow clone VB2 planted in highly contaminated soil treated with different treatments (C – control, L – quick lime, D – dolomite, S – superphosphate, P – rock phosphate). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each organ of plant.

In most of treatments (quick lime, dolomite, and rock phosphate application), an increase in Pb content in leaves and twigs was not significantly different compared to the control (see Fig. 7) as in the case of plant-available Pb concentrations in soil (see Table 1).

We observed a decreasing trend in dry production of willow after all additives application compared to the control (see Fig. 8). Nevertheless, in the cases of quick lime and superphosphate applications, a decrease in dry aboveground biomass production was not significantly different.



Figure 7. Pb content in leaves and twigs of willow clone VB2 planted in highly contaminated soil treated with different treatments (C – control, L – quick lime, D – dolomite, S – superphosphate, P – rock phosphate). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each organ of plant.



Figure 8. Dry biomass production of willow clone VB2 (leaves, twigs) planted in highly contaminated soil treated with different treatments (C – control, L – quick lime, D – dolomite, S – superphosphate, P – rock phosphate). Error bars represent standard error of the mean (SE). Using Tukey post-hoc test, treatments with the same letter are not significantly different within each organ of plant.

# Discussion

# Field experiment.

Cadmium is mobile element occurring in large portion in exchangeable and plant-available fractions. <sup>12</sup> Both willow clones accumulated more Cd in branches as compared to Pb; similar results were obtained by Vysloužilová et al.<sup>13</sup> Willows confirmed their ability as Cd accumulators, and it was also observed by Dickinson and Pulford.<sup>14</sup> On the other hand, poplars confirmed their ability as Pb accumulators, and this was also observed in study of Fischerová et al.<sup>15</sup> Lead is low mobile element. Only small portion of Pb in soil is occurred in easily mobilisable fraction. In the case of Pb, efficiency of phytoextraction was very low in medium contaminated soil "Podlesí"; similar results see also in Komárek et al.<sup>16</sup>

Cd and Pb contents in branches of fast growing trees were negatively affected after sewage sludge application. According to Chaney et al.<sup>17</sup> fertilization and other agronomic activities necessary for high biomass production can negatively affect risk elements uptake. Liming and organic fertilization can reduce mobility and availability these elements by plants. Ability of organic matter to bond risk elements can reduce phytoextraction in low and moderately contaminated soils. Quality of organic matter, especially ratio of fulvic and humic acids can play an important role in reduction of risk elements uptake by plants. Fulvic acids create soluble chelate and humic acids with high ability to bond heavy metals create insoluble complexes especially in acid conditions.<sup>18</sup> Cadmium in low content is bond in sewage sludge on fulvic acids. On the other hand, Pb is bonded especially in humic acids.<sup>19</sup>

High biomass production of fast growing trees is important medium for phytoextraction.<sup>20</sup> In our study was confirmed that risk element contents in branches and especially yield of biomass play an important role in removal of Cd and Pb by fast growing trees; it was also reported by Hammer et al.<sup>21</sup> In this study, total removal of elements from soil with high pH has similar trend as biomass production.

#### Pot experiment.

The highest decrease in plant-available Cd concentrations after quick lime application is connected with high increase in soil pH; see other studies. <sup>22, 23</sup> On the other hand, an increase in plant-available Cd concentrations after superphosphate applications is probably because of decrease of soil pH, observed also by Wang et al. <sup>24</sup> There is no positive effect on decrease of plant-available Pb concentrations after all soil additives application because of generally low Pb mobility in soil.<sup>25</sup>

There is an increasing trend in accumulation Cd and Pb contents in leaves of willow clone VB2 after first vegetation period. Nevertheless, the results were not significantly different. So it is necessary to monitore these clones of willow in the same conditions for more vegetation periods for equitable evaluating soil additives treatments. We can conclude that all tested additives (quick lime, dolomite, superphosphate and rock phosphate) negatively affected dry biomass production of willow clone VB2 after the first vegetation period in pot experiment. The results of dry biomass production were surprising. Stabilization of plantavailable concentration in soil especially after quick lime application would give good possibilities for health growth of willows8. The results were probably affected by premature leaf fall because of Fe deficiency. Therefore, it will be necessary to treat willows by Fe fertilizers during the next vegetation periods.

# Conclusion

Results of both experiments confirm that high biomass production plays a crucial role in total removal of both toxic elements from contaminated soils. Application of organic matter did not affect biomass production for longer period in field experiment but limited element uptake, with addition of additives into extremely contaminated soil was not successful in improvement biomass production, however decreased metal availability in soil.

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