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Editorial

This year the publishing of the first issue is rather late. World economic crisis is evidently influencing the writing of the contributions to the *International Journal of Sanitary Engineering Research*. Hopefully the issue is completed and published with several interesting papers.

Slovenia is known as intensive wine producing country. The area covered with the wine yards is approx. 22.000 ha or 3.9 % of the total Slovenian agriculture area. Therefore precautionary principle at using the phytopharmaceutical substances is very important in order to decrease negative impacts of the heavy metals and chemicals on the environment, especially to the groundwater and to the sediments in the rivers. V. Weingerl carried out interesting study on the influence of the use the organometallic phytopharmaceutical fertilizers containing copper and zinc on the distribution those metals in the wine yard soil and in the Drava river sediments. Several wine producers in Slovenia and abroad are introducing the biodynamic methods in wine yards protection. This is evidently interesting topics for the one of the next issues of the *Journal of the Sanitary Engineering Research.*

Small and Medium Sized Enterprises (SMEs) are generating substantial amounts of waste. The authors of the paper *Waste Treatment Within Small and Medium Sized Enterprise and Proposals for Improvement* found out that Slovenian SMEs have no enough knowledge and resources to deal with the wastes. Several years ago almost all EU countries established the National Cleaner Production Centers which have been helping companies with the waste of production and introducing the methods and technologies for the waste prevention at the source. Slovenia has not established the National Cleaner Production Center and it seems that it will never be established. So far companies themselves are obliged to find resources at solving problems related with the waste and how they are successful is evident from the presented study.

Next paper deals with the analysis of the freezing time according to the composition of the product. The food composition of food product has significant effect on freezing time considering that the products have the same shape, mass and relief. The main influence on the freezing time is the water quantity and water soluble components in the product. Predicting the freezing time is therefore logical result of this study and authors are now obliged to develop mathematical model for predicting freezing time according to the product composition and temperatures for the particular type of product.

The genetically modified organisms are in the centre of the interests of the general public, non governmental organisations, professional organisations, scientists and politics. On the one hand the world is faced with the lack of the food in less developed countries and on the other hand the developed countries have the surplus of food. In the near future almost all non renewable sources will be spent, the energy and raw materials will be produced from the biomass. The agriculture area will be divided between raw material production, energy production and food production. The prices of the food will rise and it seems that one of the answers for this challenge is the use of genetically modified organisms. Threads and opportunities of this organisms are presented in the paper *Health Risk and Genetically Modified Organisms.*

Sincerely,

Janez Petek
Editor-in-Chief

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Content

Original scientific paper

Influence of Food Composition on Freezing Time

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ABSTRACT

Freezing is still the best way to preserve food when is carried out properly and allows production of food without any chemical preservatives. It is important to accurately predict the freezing time of different kinds of food to assess the quality and safety of the foodstuffs, processing requirements, and economical aspects of food freezing. The aim of this research was to investigate the influence of food product composition on freezing time. The research based on hypothesis: the composition of food product has significant effect on freezing time considering that the products have the same shape, mass and relief. In our research we recorded four batches of standard product with three different fillings (chocolate, vanilla and forest fruit) at the same freezing conditions. The products from puff pastry were always made from the same ingredients and also mass, shape, relief, slab and surface were constant. The core temperature after 41 minutes of freezing at air temperature – 32 °C ± 2 °C and constant velocity reached in chocolate product – 24.4 °C ± 0.2 °C, meanwhile in forest fruit – 17.0 °C ± 0.2 °C and vanilla only – 12.8 °C ± 0.2 °C; thus confirmed our hypothesis. For SMEs (small and medium size food enterprisers) which represent 99 % of all enterprises in the EU, it is essential to establish their own model for predicting freezing time which is more applicable that general theoretical models that require a lot of data which are not common accessible for SMEs.

KEY WORDS:

Freezing, Food composition, Freezing time, Freezing point, Food quality, Food safety.

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INTRODUCTION

Like most food preservation methods, the freezing process for food has evolved over a significant period of time. As an extension of cooling, the primary goal of food freezing has been the reduction of microbial growth and/or control of reactions causing spoilage of the food, as well as the corresponding extension of product shelf-life. In addition, the process has extended the availability of many food commodities for consumption at any time during the year. Frozen food can be transported for longer distances and the process has contributed to making foods available on a worldwide basis. For most applications, the food products are held in storage for some period of time before thaned, and prepared for consumption. More recently, the process has evolved to include a variety of products that are consumed in a frozen state [1]. The principal objective of the new general and specific hygiene rules is to ensure a high level of consumer protection with regard to food safety. Food business operators shall ensure that all stages of production, processing and distribution of food under their control, satisfy the relevant hygiene requirements, laid down in Regulation (EC) No. 852/2004. This regulation emphasizes the importance of cold chain throughout HACCP (Hazard Analysis and Critical Control Point) system for all foodstuff that require cooling or freezing regime [2]. Kassianenko and co-workers [3] emphasis the importance of cold chain, which is a vital part of modern global trade as it has impact on all food commodities. In today's modern society, refrigerated storage is one of the most widely practiced methods of preserving perishable food. Improper use of this process increases the potential risk and microbial hazards will advance, thus leading to the food borne illnesses.

The principal objective of the new general and specific hygiene rules is to ensure a high level of consumer protection with regard to food safety.

The purposes of food freezing is preservation of food, reducing the activity of enzymes and microorganisms, reducing the amount of liquid water for microbial growth and reducing water activity of foods [2,4,5,6]. Freezing is still the best way to preserve food when is carried out properly and allows production of food without any chemical preservatives. Thermo physical properties of frozen food are used to estimate the rate of heat transfer and to calculate the heat load in process such as freezing and thawing [7]. The early calculations and analyses associated with freezing and thawing, primary used constant and uniform thermo physical properties [8]. The calculations and analyses were typically oversimplified and inaccurate. Numerical analyses such as finite difference methods were used widely to analyze thermal food processes [9]. The large number of food products, available today, create a great demand for knowledge on thermo physical properties. Since foodstuff are composite materials, the thermo physical properties are clearly a function of the components [10,11]. The core temperature for frozen food are prescribed by the Regulation upon safety of frozen food, 63/2002 [12] (the Council directive of 21 December 1988 on the approximation of the laws of the Member States relating to quick-frozen foodstuffs for human consumption (89/108/EEC)) and so it is an obligation to release the products on market with proper core temperature which has to be below – 18 °C. Nevertheless the monitoring of tempera-

Freezing is still the best way to preserve food when is carried out properly and allows production of food without any chemical preservatives.

The large number of food products, available today, create a great demand for knowledge on thermo physical properties.

ture during the transport is regulated with the Commission Regulation (EC) No. 37/2005 [13] of 12 January 2005 on the monitoring of temperatures in the means of transport; warehousing and storage of quick-frozen foodstuffs intended for human consumption and allowed short time deviation of temperature should not exceed 3 °C.

It is important to accurately predict the freezing time of foods to assess the quality and safety, processing requirements, and economical aspects of food freezing.

It is important to accurately predict the freezing time of foods to assess the quality and safety, processing requirements, and economical aspects of food freezing [14]. But freezing process has a problem with moving boundaries [15]. Food, undergoing freezing, release latent heat over a range of temperatures. Freezing does not occur at unique temperature. In addition, food do not have constant thermal properties during freezing. As result, no exact mathematical model exists for predicting the freezing time of foods [16,17].

As proposed by Cleland and co-workers in successive papers [4], one of the ways to determine the freezing time of a multi-dimensional product is to know both the freezing time of a slab under the same operating conditions and a geometric shape factor [18]. Authors initially noted such a factor, such as EHTD (the equivalent heat transfer dimensionality). Therefore, the freezing time of a food of any shape can be calculated by quite a lot of different equations and are quite difficult for use in industry because of the wide range of variables. The experimental or testing method which has a lot of process traps in often used in practice. The most often mistake is overlaying the parameters to other similar products (mass, shape, relief, slab, etc.). The freezing time requirement for each entity is estimated by the simulation model using the Plank's equation. Plank developed an equation, based on the unique phase change model, for estimating freezing time for different geometrical shapes, and allowing for varying film coefficients. The equation, derived from one-dimensional infinite slab geometry and has been analytically extended for infinite cylinders, spheres and for finite parallelepiped geometries. Plank's equation is common worldwide used and can be expressed as follows [19], equation 1. Where the t_f is time required for freezing (in min), ρ product density, ΔH latent heat of freezing, R characteristic half thickness of the food object, h, k_f are heat transfer coefficient and thermal conductivity (before freezing) respectively θ_{if}, θ_a denote the freezing point and ambient temperatures.

The freezing time of a food of any shape can be calculated by quite a lot of different equations and are quite difficult for use in industry because of the wide range of variables.

$$t_f = \frac{\rho \cdot \Delta H}{\theta_{if} - \theta_a}\left(\frac{R}{h} + \frac{R^2}{2k_f}\right) \qquad (1)$$

The most often mistake is overlaying the parameters to other similar products.

t_f	time for freezing (min)
ρ	product density (kg/m^3)
ΔH	latent heat of freezing (kJ/kg)
R	half thickness of the food object (m)
h, k_f	heat transfer coefficient (W/(m^2·C))
θ_{if}, θ_a	thermal conductivity (W/(m·C))

Carefully planned and efficiently provided education of employees is of the great importance. Lectures should be carried out by competent ex-

perts with relevant experience, who will provide specific information to every employee according to their assigned task. Employees should be personally responsible for their work [20]. The consumer is also a source of hazard for itself and that should be incorporated in the risk assessment. The results from research carried out by Ovca and Jevšnik [21] confirmed that the term "cold chain" is not well known among consumers. They also confirmed that Slovenian and European consumers place the responsibility of maintaining a cold chain into other parts of the food chain. One of the reasons for poor knowledge of the cold chain could be due to the unsatisfactory efforts of governmental and non-governmental organizations, which are responsible for educating and informing consumers.

The role of the consumer in maintaining a cold chain is more important than is currently thought, and is greater than the importance attached to other parts of the food chain.

Lectures should be carried out by competent experts with relevant experience, who will provide specific information to every employee according to their assigned task.

MATERIALS AND METHODS

Samples were prepared as part of daily production on continuous line at food enterpriser according to its production capacity. The product core temperature and time were measured during shock freezing using RT-DTEMP101; SN: M32551, for the range from -100 °C to +600 °C (MadgeTech, Contoocook, New Hampshire, USA), calibrated with measurement error 0.2 °C and evaluated using program MadgeTech Data Recorder Software, version 2.00.63. with the statistical parameters included. We recorded core temperature every 6 seconds and thus we got for each measure 410 thermal points for describing the decrease of temperature in core of product. Water activity was measure using Testo 650 with electrode 0628-0044 (910) at 25 °C, meanwhile the compositions were analyzed in Slovenian National Public Health Institute. The results of analyses are stated in Table 1. The puff pastry was always made from the same ingredients; also mass, shape, relief and surface were constant. Times of freezing, air temperature, air speed in continuous shock freezer were also constant and were not changed during the process. Freezing medium was always cooled air with temperature of -32.0 °C ± 2.0 °C, constant velocity and time of freezing for 41 minutes. Selected fills were vanilla, chocolate and forest fruits. Each piece of product was weighted on continuous packaging equipment (n = 9000; 100.00 g ± 2.8 g). We measured the temperature of each type of product in for parallel batch at the same conditions: (vanilla, n = 4; chocolate, n = 4; forest fruits, n = 4). Predicted freezing time for mass 100 g was 40 min – 45 min at - 32.0 °C ± 2.0 °C, constant velocity, as stated in producer's instructions. The time of freezing is managed by belt speed. At one time in freezer were 820 kg of product or 8,200 pieces. The results of core temperature after 41 minutes of freezing were processed by the XLSTAT 2009.6.01 Comparison of samples using nonparametric test, multiple pair wise comparisons using the Nemenyi's procedure/Two-tailed test with significance level 5 %.

The role of the consumer in maintaining a cold chain is more important than is currently thought, and is greater than the importance attached to other parts of the food chain.

RESULTS AND DISCUSSION

According to our four batches we recorded core temperature every 6 seconds and thus we got for each measure 410 thermal points for describing the decrease of temperature in core of product. When we compare the data between four parallels within one type of product we ascertain no statistical difference ($p < 0.001$). Results from recorded data for all products are stated on the Figures 1 from *a* to *d*. Within one type of product there is no difference within type of product or according of the used fill. As Figure 1 shows that the time of freezing was constant 41 minutes and at this time only one product (chocolate filling) reached the temperature below -18 °C and the core temperature after 41 minutes of freezing reached -24.4 °C ± 0.2 °C. The core temperature for product with forest fruit filling almost reached the required temperature (-18 °C) but nevertheless it was still with its -17.0 °C ± 0.2 °C more than 1.0 °C to high. The highest core temperature we measured in product with vanilla filling and it was -12.8 °C ± 0.2 °C after 41 minutes of freezing. Summary data of four investigated batches are shown in the Table 2. Reason for such differences between similar products is in the specific heat and enthalpy. In general we can conclude that the food with higher content of water have higher specific heat and higher enthalpy (at 0 °C) thus need longer freezing time compare to similar prod-

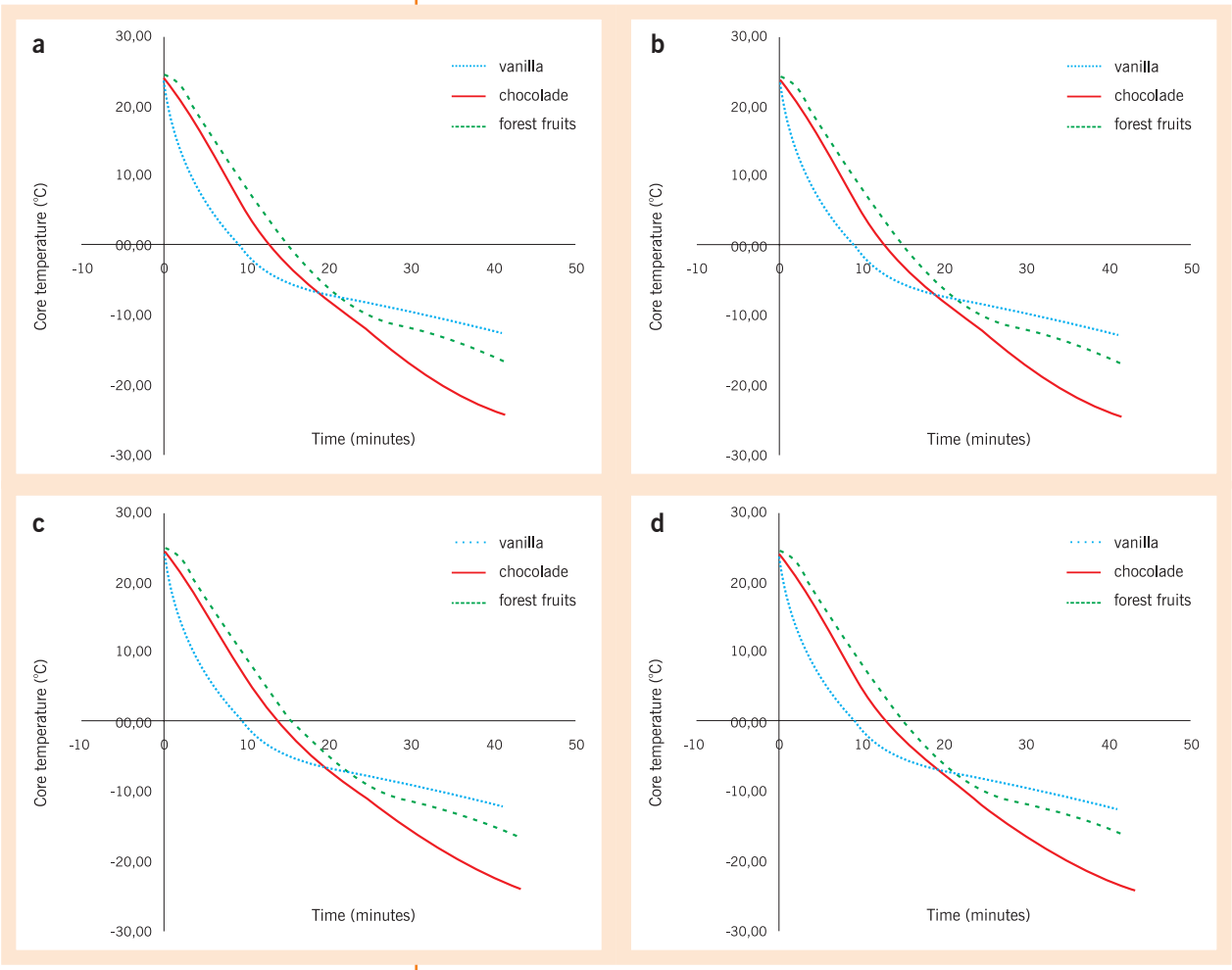

Figure 1.
Core temperature in correlation to time of freezing for four batches.
a. the first batch; **b.** the second batch; **c.** the third batch; **d.** the fourth batch.

Table 1:
Composition of food product with three different types of filling.

	C filling	V filling	FF filling	C	V	FF
Proteins	8.15	1.12	1.79	6.17	5.73	5.11
Carbohydrates	56.21	30.86	51.86	45.02	32.89	44.28
Fat	33.52	1.68	1.74	27.24	12.96	18.19
Water	1.08	65.32	43.56	20.5	47.45	31.4
Ash	1.04	1.02	1.05	1.07	0.97	1.02
a_w (25 °C)	0.394	0.972	0.946	0.879	0.952	0.917

C, V, FF with postscript "filling" are data for pure fill, V: vanilla product, C: chocolate product, FF: forest fruit product, a_w water activity.

Table 2:
Summary of recorded core temperatures in food product with three different types of filling.

	t (min.) product	0	5	10	15	20	25	30	35	40
1st	V	23.4	15.9	-1.2	-5.1	-7.1	-8.4	-9.5	-11.2	-12.8
	C	23.9	15.9	4.8	-3.2	-8.3	-12.6	-16.5	-21.1	-24.3
	FF	24.4	15.9	7.5	-0.6	-6.2	-10.4	-11.8	-13.7	-16.9
2nd	V	23.9	15.9	-1.3	-5.3	-7.1	-8.5	-9.5	-11.2	-12.7
	C	23.9	15.9	4.7	-3.4	-8.4	-12.5	-16.6	-21.2	-24.3
	FF	24.4	15.9	7.4	-0.5	-6.3	-10.4	-11.8	-13.9	-16.9
3th	V	23.4	15.9	-1.4	-5.4	-7.0	-8.4	-9.6	-11.2	-12.8
	C	24.1	15.9	4.5	-3.7	-8.3	-12.6	-16.7	-21.3	-24.3
	FF	24.4	15.9	7.3	-0.5	-6.4	-10.4	-11.8	-13.8	-16.9
4th	V	24.1	15.9	-1.4	-5.5	-7.1	-8.5	-9.6	-11.2	-12.8
	C	24.0	15.9	4.4	-3.5	-8.4	-12.7	-16.7	-21.3	-24.4
	FF	24.4	15.9	7.2	-0.7	-6.5	-10.5	-11.9	-13.9	-17.0
$\bar{x}$	V	24.1^a	15.9^a	-1.4^c	-5.5^c	-7.1^b	-8.5^a	-9.6^a	-11.2^a	-12.8^a
	C	24.0^a	15.9^a	4.4^b	-3.5^b	-8.4^c	-12.7^c	-16.7^c	-21.3^c	-24.4^c
	FF	24.4^a	15.9^a	7.2^a	-0.7^a	-6.5^a	-10.5^b	-11.9^b	-13.9^b	-17.0^b
p-value		$p > 0.05$	$p > 0.05$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$

t: time of freezing in minutes, V: vanilla product, C: chocolate product, FF: forest fruit product, $\bar{x}$: average values for four batches, Significant at $p \leq 0.001$; significant at $p \leq 0.01$; significant at $p \leq 0.05$; $p > 0.05$, not significant, Values followed by a different letter are significantly different along the column for the Duncan (0.05) test.

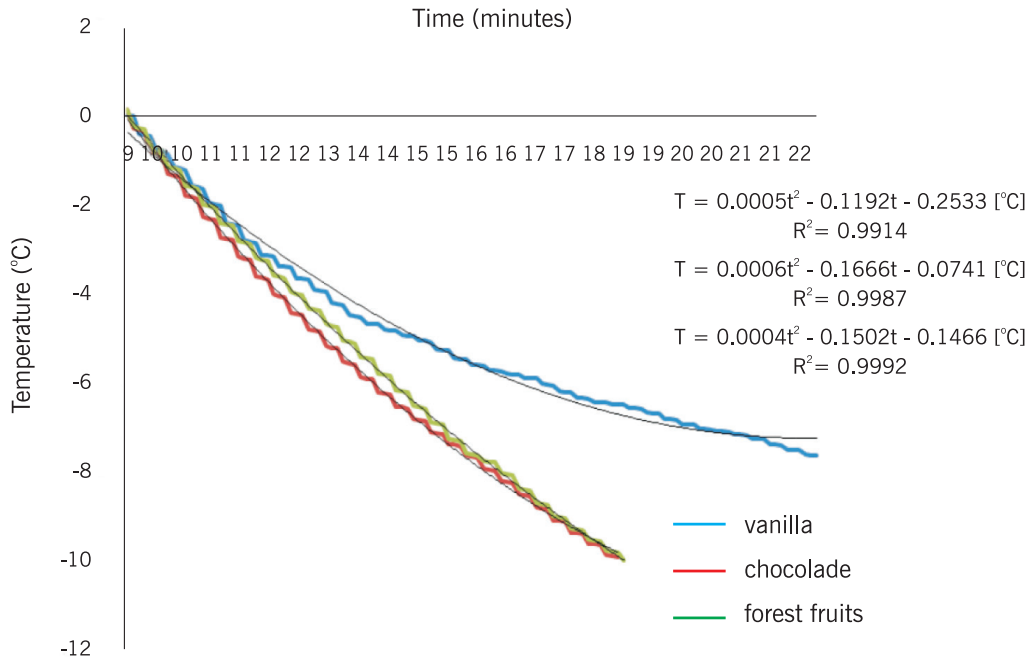

Figure 2.

Phase change from liquid to solid (ice).
T: predicted temperature (°C);
t: time (min) in range from 0 to -12 °C.

ucts with lover water content. Freezing time as correlation to water content as well as dry matter from our research is described with equation on the Figures 2 and 3.

There are exceptions like cucumber which has water content 95.4 % and enthalpy 390 kJ/kg (at 0 °C), but low specific heat capacity (1.02 kJ/(kg·K)) thus its water in 100 % freeze at -5 °C, meanwhile tomato pulp with water content 92.9 % and enthalpy 382 kJ/kg (at 0 °C), but higher specific heat capacity (4.02 kJ/(kg·K)) at -5 °C have still 16 % of *unfrozen water* [11]. The time of cooling when the 0 °C was

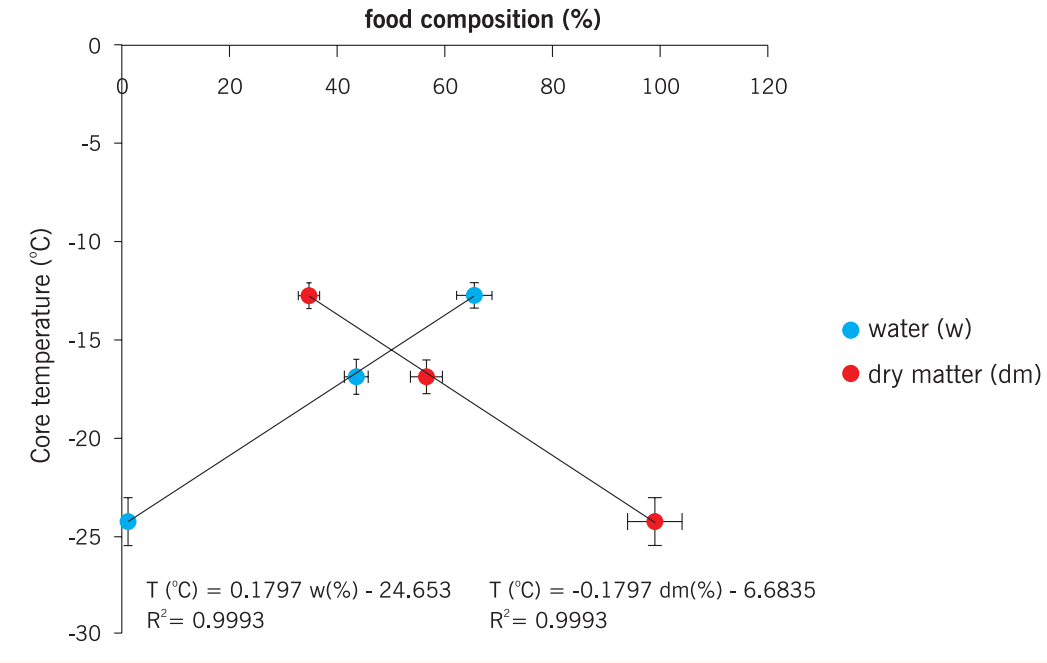

Figure 3.

Food composition in correlation to core temperature in sampled products.

achieved, was also different – 9 minutes for vanilla, 13 minutes for chocolate and 15 minutes for forest fruit. So the filling with the highest content of water cool down to 0 °C rapidly compare to other two. The cooling gradient from 24 °C to 0 °C for vanilla was 2.7 °C/min, 1.8 °C/min for chocolate and 1.6 °C/min for forest fruit filling. This temperature cooling gradient is also shown on the Figures 1 to 3. Although any solid food is not solution, the aqueous component in the frozen solid food can be considered as a mixture of ice and a solution of nonaqueous components in liquid water. As water in the food freezes into pure ice during the freezing, the remaining solution becomes more and more concentrated. Thus, depression of the freezing point of the food occurs and continues as concentration increases. The net effects of dynamic freezing point depression are that the initial freezing point of the food is below 0 °C. The freezing process occurs over a range of temperatures, which is different from the freezing process of pure water at unique temperature. The phase changes from liquid water to solid (ice) are shown in the Figure 2.

Although any solid food is not solution, the aqueous component in the frozen solid food can be considered as a mixture of ice and a solution of nonaqueous components in liquid water.

When the 0 °C was reached after different time in each product, the temperature gradient in all products changed rapidly because of the changing of physical state from liquid to solid (ice). At this point the phenomena discussed above appear, so the products with higher content of water need more energy to convert all water to ice. The product with chocolate filling which has the highest content of fat continues to cool down at the quite the same temperature gradient. It can be assumed that the fat in the chocolate consist majority from saturated fatty acids which are solid at room temperature, thus there is less water than in other two cases that has to be converted from liquid to solid.

The products with higher content of water need more energy to convert all water to ice.

CONCLUSIONS

As expected, the product containing the highest level of water (vanilla filling) has not reached the target temperature and it was with the core temperature extremely above the -18 °C and thus can represent a food safety hazard as well as risk for consumers. There are data confirming microbial growth of some Gram-negative bacteria at -4 °C and growth of bacilli, that is, Gram-positive bacteria at -7 °C [4]. The problem of higher core temperature than -18 °C in frozen food products, which is also obligatory by the law, is not concerning only the primary production process or enterprisers but concerns all actors in food supply chain [2].

In general we need longer time of freezing for foodstuff with high water and the water soluble components.

The data of food composition as well as shape, density, surface are necessary to predict freezing conditions. On one hand it is possible to reach target temperature by pre-cooling of raw material (where possible), while on the other hand we can prolong the time of freezing and lower the temperature of freezing media (air) and increase its velocity for better heat transfer (not always applicative). In general we need longer time of freezing for foodstuff with high water in the water soluble components. Because of the effect of lowering freezing point as result of concentrating the solution in food, as well as knowing the mean of the importance of specific heat. There are also some other methods to

In practice every part of food supply chain has the manage system which pushes the action to limits like just in time management.

decrease freezing time such as use of cryogenic freezing, where extreme low temperatures are used by liquid gasses (N_2, He, CO_2) or increasing the dry matter by adding hydrocolloids, proteins and sugars.

Global food safety will be achieved, when every single link in the food chain will entirely (in its indoor and outdoor environment) become master of its particular area and will trust in activity of both previous and following link in the food safety circle "from farm to table", not ignoring consumer [22]. Usually in practice every part of food supply chain has the manage system which pushes the action to limits like just in time management. So there is possibility that frozen food with inadequate temperature from enterpriser over the logistics, reach the retailer shelves thus even consumer, who can storage such a frozen food according to expire date. For SMEs is essential to establish their own model for predicting freezing time which is more applicable than general theoretical models that require a lot of data which are not common accessible for SMEs.

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Original scientific paper

Copper and Zinc Accumulation in Vineyard Soils Treated with Cu and Zn Containing Phytopharmaceuticals

Vesna **WEINGERL**

ABSTRACT

Long term use of copper and zinc containing phytopharmaceuticals in vineyard areas causes increased accumulation of total Cu and Zn in the surface layer of soil. Copper and zinc accumulation and distribution in vineyard soils is discussed as a step towards understanding Cu and Zn existence in soils and their availability to plants. Several single extractions were performed to the determination of total Cu and Zn content after aqua regia digestion. The use of Cu containing phytopharmaceuticals has increased the total Cu concentration in analyzed vineyard soils to 97 mg/kg compared to background levels of approximately 30 mg/kg.

The amounts of plant available Cu and Zn extracted by the EDTA ranged as follows, when expressed relative to the total Cu or Zn content in the soils: 13.6 % – 40.4 % for Cu and 3.5 % – 11.6 % for Zn.

The study shows that copper stays active in soils longer than ten years, and may result in leaching and can transfer to deeper soil layers and consequently to ground or the surface waters.

KEYWORDS:

Vineyard, Soil, Copper, Zinc, Phytopharmaceuticals.

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INTRODUCTION

In order to control vine (*Vitis vinifera L.*) diseases, such as downy mildew caused by *Plasmopara viticola*, different phytopharmaceuticals, especially Cu-based ones, have been applied extensively to vineyards. The application of these phytopharmaceuticals ($CuSO_4$ + $Ca(OH)_2$); Cu_2O), including the Bordeaux mixture $CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$ and more recently copper oxychloride $CuCl_2 \cdot 3Cu(OH)_2$ and hydroxide ($Cu(OH)_2$), resulted into increased Cu concentrations in vineyard soils. The content of Cu significantly increases with a vineyard's age.

Heavy metals are the main pollutants which accumulate in vineyards´ ecosystems. Because of viticulture intensive practice, the phytopharmaceuticals and fertilizers are the main source of metal pollution. While the soil purifies slowly and partially, metal pollutants tend to accumulate [1].

Heavy metals are the main pollutants which accumulate in vineyards´ ecosystems.

In many regions of the world there is a concern about the risks that the accumulation of copper in agricultural soils may pose to human health, ecological health, and the long-term sustainability of agricultural lands. The phytotoxic effects of copper to plants have been widely studied [2,3], but it is not expected that copper causes phytotoxicity in mature grapevines as they tend to have deep root systems that extend below the surface soils where most of the applied copper tends to remain.

The impacts on soil biology and implications for soil fertility are more of the concern in established vineyards. Several studies have shown the elevated copper concentrations to adversely affect the fertility of vineyard soils. Increased Cu concentrations in soil (more than 80 mg/kg) reduce earthworm abundance; therefore, any harm to earthworm populations could affect the productivity and efficiency of vineyards [4].

The impacts on soil biology and implications for soil fertility are more of the concern in established vineyards.

In response to environmental concerns over the use of copper fungicides such as the accumulation of copper in agricultural soils and the potential impact on soil ecology, regulators in some European countries have imposed restrictions on the use of copper-based fungicides. For example, copper use has been banned in The Netherlands, and Switzerland has restricted the amount of copper that can be applied per hectare [2].

Micronutrient elements like Cu and Zn form insoluble compounds and also bond strongly to mineral particles and organic matter. Considering availability to vines, only a nutrient in the soil solution is immediately available to the vines. Deficiency occurs when a nutrients supply is unsufficient to meet a vines demand; conversely, when supply exceeds demand or a nonnutrient element accumulates in the root zone, toxicity may occur.

Micronutrient elements like Cu and Zn form insoluble compounds and also bond strongly to mineral particles and organic matter.

In previous research main focus has been pointed out to Zn determination in vineyard soils. Zinc additions in the Orlica vineyard soil in Meranovo were much larger than uptake; the consequence was accumulation of zinc in the soil. The analysis of the Meranovo stream sediment which springs in the Pohorje territory and runs below the vineyard to the river Drava showed much lower zinc concentrations in the stream

Zinc is hard to wash away, therefore the contents of zinc are the highest in the surface layer.

sediment before the stream reaches the vineyard and than after it leaves the vineyard. The increasing zinc content in the sediment is caused by the use of zinc containing phytopharmaceutical substances and mineral fertilizers [5].

Mobility of copper and zinc in soil is closely connected to soil reaction. With increased acidity of the soil there is also the increase of the quantity of element available to plants. The content of zinc is considerably lower in acid than in alkaline soil. Zinc is hard to wash away, therefore the contents of zinc are the highest in the surface layer [6,7].

Contents of copper, zinc and other heavy metals in Slovene soil on the country area were researched by Andjelov and Šajn [8,9]. Increased contents of copper and zinc were established in the Carinthia area (the Mežica mine), in Celje area (the Celje zinc processing plant) and in the areas of intensive plantation production of fruit and grape (organometallic phytopharmaceutical pesticides and fertilizers).

High levels of phosphorous, zinc, iron, manganese and aluminum may also restrict copper absorption by roots.

Micronutrients present the limitation of growth, development and consequently production of vine. Considerable deficiency as well as larger surplus of specific nutrients can be detected on vine by visual inspection. Zinc deficiency in vine occurs in the situation where soil is rich with phosphorus, phosphate ions surrounded the roots and cause the change of zinc ions to insoluble zinc phosphate when contacting the roots. The surplus of phosphate ions deactivates zinc ions into insoluble zinc phosphate as well. High phosphorus content in vineyard soil samples is caused by use of mineral fertilizers containing phosphorus (superphosphate, Thomas phosphate, complex NPK fertilisers). Reports of copper deficiency in vineyards are rare. More common are reports of copper toxicity. Copper is an essential element for living organisms, as a constituent of several enzymes and as a redox catalyst in a variety of metabolic pathways. At high concentrations, however, copper inhibits growth and interferes with several cellular processes, including photosynthesis, respiration, enzyme activity, pigment and protein synthesis and cell division [22]. Copper is strongly bound to organic matter. Peat soils and mineral soils with high levels of organic matter (6-10%) are most likely to be deficient in plant available copper. Copper availability is reduced as pH increases to 7 and above. High levels of phosphorous, zinc, iron, manganese and aluminum may also restrict copper absorption by roots. Copper is an important constituent of many proteins like ascorbic acid oxidase, cytochrome oxidase, diamine oxidase, and polyphenol oxidase. Copper is an important nutrient for many microbes. It controls molds and often alleviates perceived zinc deficiencies. Copper interacts with iron and manganese [23].

Predicting the mobility of heavy metals in soils requires the mathematical models that accurately describe metal adsorption in the presence of competing cations. A reaction-based sorption model was developed using a combination of nonspecific cation exchange reactions and competitive sorption reactions to sites with high affinity to heavy metals. The proposed model describes competitive sorption and transport of Cd, Zn, and Ni in pH range between pH 4.6 and 6.5 [8].

Heavy metal extraction efficiency depends on the combination of many factors, such as the liability of heavy metals in soil, the concentration and nature of the chelant, pH and the properties of the soil matrix. Raising soil pH is a prevention mechanism for elution of heavy metals. Chelant assisted soil washing has been widely studied for remediation of contaminated soil. A number of chelants have been tested. For soils contaminated with Pb, Zn, Cu and Cd, ethylenediamine tetraacetate (EDTA) has often been found to be the most effective [13].

The study of transition metals (Cd, Co, Cu, Mn, Mo, Pb and Zn) in the soils of the sub-Mediterranean winegrowing areas in Slovenia confirmed that the main source of transition metals pollutant in the region is intensive viticulture practice, especially the use of copper substances [10,11].

The study of transition metals in the soils of the sub-Mediterranean winegrowing areas in Slovenia confirmed that the main source of transition metals pollutant in the region is intensive viticulture practice, especially the use of copper substances.

The object of this research was to investigate the influence of organometallic phytopharmaceutical fertilizers use on copper and zinc dynamic and distribution in vineyard soil. With the use of the control soil samples, differentiation of natural and anthropogenic soil enrichment in heavy metals in wineyard areas, was possible. Content of Cu and Zn in a control sample gives natural soil profile of these two elements in soil, including the atmospheric influence. Total element contents, EDTA- and AL- (ammonium lactate) extractable (plant available) Cu and Zn with flame atomic absorption spectrometry (FAAS) method was determined. Contents of Cu and Zn were determined in wine, produced on vineyard areas, included in research. Weight balance was calculated from the data on copper content in wine (Cu uptake) and content of copper in used phytopharmaceutical substances (Cu addition). In addition, content of Cu in the sediment of the stream at a foothill of the vineyard Orlica was measured. The sediment of the stream was sampled on several sites from the spring to the river Drava. Increased copper concentration in the stream sediment was expected as the evidence of the long term use of the phytopharmaceutical substances containing copper.

METHODS

Soils of two vineyards from both riverbanks of the river Drava near Maribor, Slovenia, were sampled. Surface of the Orlica vineyard in Meranovo is 50,000 m^2 with 17,800 vines. Surface of the Kalvarija vineyard is 45,000 m^2 with 15,000 vines. Upper part of the vineyard Kalvarija (soil sample named Kalvarija 2) and lower part of the vineyard Kalvarija (soil sample named Kalvarija 3) were sampled and analysed separately. The particular vineyards were chosen because of their diversity in acidity of soil – vineyard Orlica possesses acid soil whereas Kalvarija possesses alkaline soil. Quite different content of free carbonates in vineyardsoil Kalvarija was the reason to separate this vineyard in two parts regarding to the altitude. Control soil sample was sampled above the two vineyards on uncultivated surfaces. The distance between sampling sites was approximately 10 m. In all vineyards, soil samples were collected as close to the vine lines as possible and in the middle of the row, between two sampled lines. The samples were sampled with metal probe (2 cm diameter) at depth of 0-20 cm and 20-40 cm. Two lay-

The distance between sampling sites was approximately 10 m.

We sampled according to ÖNORM L1057 which regulates soil samples taken in vineyards and orchards.

Soil samples were first dried in the air to the constant weight. Roots and larger stones were removed from the dried samples. Samples were ground in the mill and passed through 1 mm sieve.

ers were sampled to assess the distribution of Cu and Zn with depth. We sampled according to ÖNORM L1057 which regulates soil samples taken in vineyards and orchards [14].

Soil samples were first dried in the air to the constant weight. Roots and larger stones were removed from the dried samples. Samples were ground in the mill and passed through 1 mm sieve. Humidity was determined by weighing 10 g of air-dried sample into paper bag and dryed at 60 °C in a drying machine to the constant weight. Contents of Cu and Zn were calculated according to the dry weight of the sample.

Copper and zinc were determined by flame atomic absorption spectrometer, SpectrAA-10 (Varian, Mulgrave, Australia) in air/acetylene flame. Zinc absorption was measured with the use of deuterium lamp for background correction at 213.9 nm and copper absorption at 324.7 nm wavelength. Soil samples were digested in PFTE (polytetrafluoroethylene) containers in MDS 2000 microwave oven produced by CEM (CEM, Matthews's n. c., USA).

Phosphorus concentration was determined by spectrophotometric ammonium molybdate method on Varian Cary 1E spectrophotometer (Varian, Mulgrave, Australia) at 659 nm wavelength. For measuring pH in solution, Iskra MA 5750 pH meter was used (Iskra, Horjul, Slovenia) with HEC 0101 combined glass electrode.

Microwave-assisted digestion of soil samples with aqua regia: 0.5000 g ± 0.0001 g of air-dried soil sample were weighted in PTFE container and 16 mL of aqua regia (HNO_3 and HCl, 1+3) were added. The containers were covered and puted in the microwave oven. Heating programme for 12 containers was following: 1 min at 180 W, 4 min at 480 W and 60 min at 650 W. Containers were cooled to room temperature. Contents were poured through wrinkled filter paper (MN 6191/4) into 50 mL volumetric flask, deionized water were added to the mark. Contents of Cu and Zn in filtrate were determined by the FAAS method in air/acetylene flame. Calibration curve was prepared with standard solutions, added the same amount of aqua regia as the samples. A blank sample was prepared for controlling acid purity.

Total microwave-assisted digestion of soil samples: in PTFE container for digestion 1.0000 g ± 0.0001 g of air-dried soil sample were weighted, 10 mL of deionized water, 5 mL of HNO_3, 2 mL of HCl and 3 mL of HF were added. The containers were covered and put in the microwave oven.

The pressure control was programmed on $6.9 \cdot 10^5$ Pa. Twelve containers were heated for 60 min at 650 W. The containers were cooled to room temperature and the content was poured through wrinkled filter paper into 50 mL PE volumetric flask. Deionized water was added to the mark. Cu and Zn contents in the filtrate were determined by FAAS method in air/acetylene flame.

The calibration curve was prepared with standard solutions added the same amount of acids as to the samples. Blank sample was prepared for acid purity control.

Extraction of soil samples in EDTA-solution: 10.000 g ± 0.001 g of fine air-dried soil sample were weighed to 250 mL plastic container and poured over by 100 mL concentration of EDTA-solution (0.05 mol/L). Preparation of EDTA-extraction solution is described in literature [5]. The plastic container was sealed and shaken on horizontal shaker for two hours with 180 min^{-1}. After the shaking process the suspension was filtered through wrinkled filter paper. At first a few mL of the filtrate were discharged. Contents of Cu and Zn in the filtrate were determined by the FAAS method.

Soil sample extraction with AL-solution: into plastic container with cover 5.000 g ± 0.001 g of soil sample were weighted and 100 mL of AL-extraction solution was added. The container was covered and shooked for two hours on rotary shaker at 35 min^{-1}. Suspension was filtered through dry wrinkled filter paper of medium porosity (white strip). The first few mL of the filtrate were discarded. Concentration of phosphorus in the filtrate was determined by measuring molecular absorption at 659 nm wavelength in air/acetylene flame. AL–extraction solution was used as a blank sample. Preparation of AL-solution is described in literature [5]

Soil acidity was determined according to ÖNORM (16), free carbonates by Scheibler [15].

RESULTS AND DISCUSSION

In our research the main focus was pointed to the copper and zinc determination in vineyard soils. Copper addition in the Orlica vineyard soil in Meranovo is much larger than uptake, the consequence being accumulation of Cu in soil.

Vineyard soils polluted with heavy metals present a serious environmental concern. Copper and zinc originating from the intensive application of Cu- and Zn-based phytopharmaceuticals belongs to the most important contaminants of the vineyard soils. Both elements can migrate through the soil layers in vineyard and pose thus an important risk for groundwater quality.

Detection and quantification limits for total and extractable copper determination methods in soil samples are shown in the Table 1.

To verify the accuracy of total Cu, standard reference material 2709 (San Joaquin Soil, National Institute of Standards and Technology) was analysed. Comparison of our results with certified values in SRM is given in the Table 2.

It is evident from the Table 1 and Table 2 that sensitivity, precision and accuracy of analytical values were satisfactory.

Considering pH soil value, the particular vineyards were chosen for their diversity in acidity of soil. Vineyard Orlica possesses acid soil whereas vineyard Kalvarija possesses alkaline soil. Control soil samples were sampled above the two vineyards on uncultivated surfaces. Acid soil reaction is caused by the lack of exchanging metal ions. Cu and Zn con-

Copper addition in the Orlica vineyard soil in Meranovo is much larger than uptake, the consequence being accumulation of Cu in soil.

Detection and quantification limits for total and extractable copper determination methods in soil samples are shown in the Table 1.

Table 1:
Linear ranges, detection and quantisation limits for determination of Cu by FAAS and P_2O_5 by MAS.

	Linear range (mg/L)	Detection limit (mg/L)	Quantisation limit (mg/kg)
Cu – total	0.2 – 1.0	0.1060	10.6
Cu – EDTA	0.5 – 5.0	0.0350	3.5
Cu – AL	0.5 – 5.0	0.0300	2.8
P_2O_5	0.1 – 4.0	0.0002	30.0

Table 2:
Precision of total Cu content determination in SRM 2709.

	Mean conc.* (mg/kg)	RSD (%)	Certified value (mg/kg)	Measured value (mg/kg)
Cu	34.6	1.9	34.6 ± 0.7	34.6 ± 1.1

*Mean concentration, result of ten determinations of the same sample.

tent therefore much lower in acid that in alkaline soil. Results of comparison between total copper and zinc content in the vineyard soil samples and control samples to pH value of the samples are shown in the Figure 1.

The use of Cu containing phytopharmaceuticals has increased to total Cu concentration in analysed vineyard soil, 97 mg/kg, compared to background levels of approximately 30 mg/kg.

Content of the Zn increases with soil pH value in all analysed vineyard soil samples and control samples. Content of the Cu trended increasing

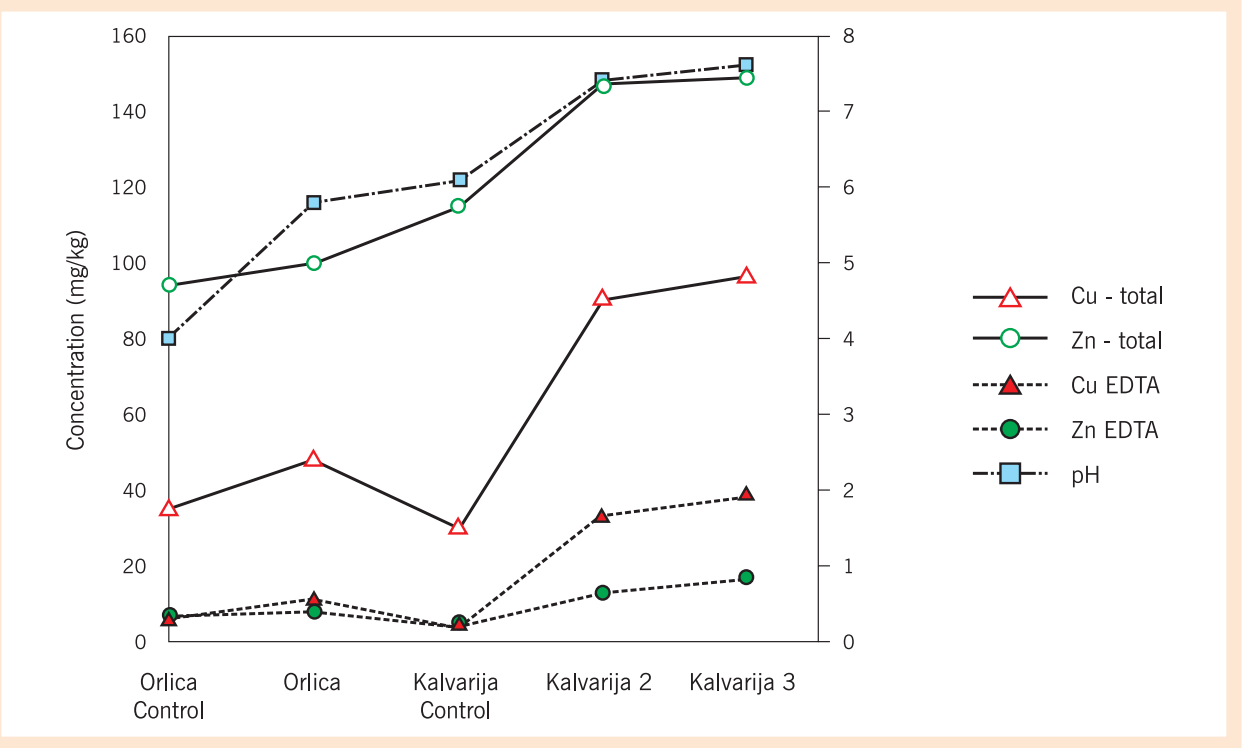

Figure 1.
Correlation between total and EDTA extractable Cu and total and EDTA extractable Zn content (amounts in mg/kg on primary axis) and pH soil value (amounts on secondary axis).

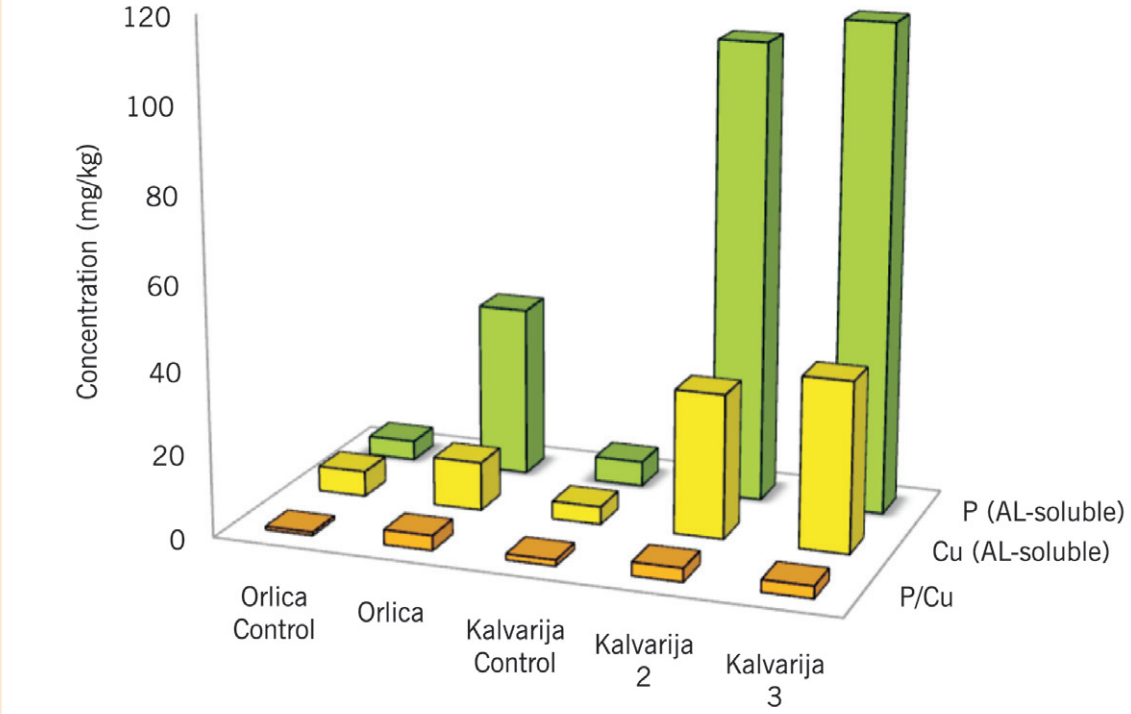

Figure 2.
Content of AL-soluble copper and phosphorus, and P/Cu proportion in soil samples.

with pH soil value with one exception – the control soil sample from Kalvarija vineyard area. Mobility of copper and zinc in soil is closely connected to soil reaction. With increased pH value of soil there is also an increase in the quantity of element available to the plants. Contents of copper and zinc are considerably lower in acid than in alkaline soil.

Amounts of plant available Cu and Zn extracted by the EDTA ranged as follows, when expressed relative to the total Cu or Zn content in the soils: between 13.6 % and 40.4 % for copper and between 3.5 % and 11.6 % for zinc.

Proportion between AL-extractable phosphorus and copper content in vineyard soil samples and control soil samples is shown in the Table 3 and graphically in the Figure 2.

It is evident from the Figure 2 that there is obvious difference between amounts of EDTA extractable copper and phosphorus in vineyard soil samples (Orlica, Kalvarija 2, Kalvarija 3) and amounts of the same elements in control soil samples (Orlica control, Kalvarija control). Concentrations of AL-soluble phosphorus are up to nineteen times higher in vineyard soils than in control soil samples (uncultivated surfaces). Phosphorus and copper proportion is 2 to 4 times lower in control soil samples than in intensively cultivated soil samples.

Addition and uptake of copper in the Orlica vineyard was also calculated. Annual copper addition was calculated from copper content data in phytopharmaceutical substances used for vineyard protection in one-year period. The data are shown in the Table 4.

Annual copper addition with phitopharmaceuticals amounts to 8.5 kg per vineyard surface which is 1.7 kg of Cu per ha. Since 1996 the vine-

Table 3:
Phosphorus and copper content in AL- extracts from soil samples and P/Cu proportion.

Soil sample	P (AL-soluble) (mg/kg)	Cu (AL-soluble) (mg/kg)	P/Cu Mass proportion
Orlica control	4.6	6.0	0.8
Orlica	40.6	11.5	3.5
Kalvarija control	5.9	4.2	1.4
Kalvarija 2	109.1	34.2	3.2
Kalvarija 3	115.4	40.4	2.9

Table 4:
Content of Cu in phytopharmaceutical substances used for Orlica vineyard protection.

	Dosage (kg)	Cu (%)	Cu (kg)
Cu – Antracol	40	17.5	7
Cu – Euparen	10	15	1.5

yard Orlica is not treated with the mineral fertilizers. Annual Cu uptake was calculated from contents data in the Sauvignon wine and than the amount of wine produced in the Orlica vineyard. Content of Cu in wine was determined by flame atomic absorption method. The wine contained 0.05 mg Cu per liter.

Determination of Cu in pomace and the rest of the vine may count significantly for total uptake of Cu from vineyard soil.

Estimated quantity of wine produced in the vineyard Orlica is 24,000 L meaning that our copper uptake with wine from this vineyard is 1.2 g per year. It is important to highlight that with wine analysis the total uptake of copper from soil could not be included. Determination of Cu in pomace and the rest of the vine (leaves, shoots …) may count significantly for total uptake of Cu from vineyard soil.

Another consideration is transfer of copper caused by rain to the nearest surface waters. Sediment of the Meranovo stream which springs in the Pohorje territory above the vineyard Orlica, and runs below the vineyard to the river Drava, was analysed. Cooper and zinc contents in the sediment are shown in the Table 5. Copper content in the Orlica vineyard soil was 48 mg/kg, zinc content was 99 mg/kg.

Due to the fact that Cu is strongly immobilized by soil organic matter and Fe, Mn, Al-(hydr)oxides, elevated Cu concentrations originating from phytopharmaceutical applications were observed in superficial ho-

Table 5:
Copper and zinc content in the Meranovo stream sediment.

Sampling site	Zn (mg/kg)	Cu (mg/kg)
Spring	96	37
Below the vineyard	95	51
Behind the vineyard	120,5	89
Mouth of the Drava	130	107

Table 6:
Limit, warning and critical imission values for Cu and Zn in soil.

	Limit imission value (mg/kg)	Warning imission value (mg/kg)	Critical imission value (mg/kg)
Cu	60	100	300
Zn	200	300	720

rizons of vineyard soils. The highest Cu concentrations were found in the surface layer (0 cm – 20 cm).

Concentrations of Cu and Zn in the stream sediment are much lower before the stream reaches the vineyard than after it leaves it. Increased concentrations of both elements are a consequence of spraying the vineyard with zinc and copper containing phytopharmaceutical substances.

With analysis of the stream sediment which springs in the Pohorje territory and runs below the vineyard Orlica to river Drava can be inferred that the increasing Cu and Zn contents in the sediment were caused by the use of phytopharmaceuticals containing those two elements. Rain washes away the vineyard soil and the silt gathers in streams. Amounts of copper in stream sediment behind the vineyard Orlica and in the mouth of the Drava River exceed the limit imission value for Cu in soil. Contents of zinc in soil and stream sediment do not exceed limit emissions.

Regulation about limit, warning and critical imission values of hazardous substances in soil deals with heavy metals in soil in Slovenia (Official gazette of the Republic of Slovenia, Vol. 68/96). Limit, warning and critical imission values for copper and zinc in soil are set out in the Table 6.

The copper amounts in vineyard Kalvarija exceed the limit imission value for Cu in soil for both parts of the vineyard. Zinc concentrations were not exceeded in discussed vineyards soils. Amounts of copper in stream sediment behind the vineyard Orlica with 89 mg/kg and in the mouth of the Drava River with 107 mg/kg exceed the limit imission value for Cu in soil.

There is a much higher portion of available copper in the vineyard soils relative to the control soil samples; there is no overall relationship between total and available copper in soils. Concentrations of plant available copper in soils cannot be predicted on the basis of total copper concentration, which is important for developing environmental guidelines.

There is a much higher portion of available copper in the vineyard soils relative to the control soil samples; there is no overall relationship between total and available copper in soils.

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Professional paper

Waste Treatment within Small and Medium Sized Enterprises and Proposals for Improvement

Katarina **ŽELEZNIK LOGAR***

ABSTRACT

Small and medium-sized enterprises (SMEs) play an important role in many economies around the world and are vitally important for a health dynamic market economy. Collectively, SMEs also cause significant impacts on the environment by their activities, products and services. The present situation, when SMEs are unaware of their environmental impact, can pose an important threat not only to the environment but also can have a negative effect on worker's health and safety. The main research questions of our study were: do entrepreneurs performing craft activities, have the necessary knowledge to carry out their waste management activities and environmental performance in general, and what possibilities for better waste treatment, waste minimization and reduction can be offered to SMEs and how? The results supported this hypothesis are that the level of knowledge about their responsibilities according to the regulations among many SMEs is still low. In the future, it will be crucial that public authorities and other key players support SMEs in increasing their efforts for improvement.

KEYWORDS:

Craft, Environmental impact, Small and medium-sized enterprises (SMEs), Waste, Waste management.

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INTRODUCTION

A great share of the businesses in Slovenia and European Union is small, representing some 99 % of all enterprises, 57 % of economic value added and about 66 % of total employment. Based on the definitions micro, small and medium-sized enterprises is made up of enterprise are which employ fewer than 250 persons and which have an annual turnover not exceeding 50 million Euro, and/or an annual balance sheet total not exceeding 43 million Euro [1].

The total environmental impact of small firms is not known either at national or regional levels, but according to some surveys about 70 % of all industrial pollution is contributed by SMEs.

The Law of Craft defines that a craft activity is a profit oriented activity being divided into craft activity, activity similar to craft and activity of home craft and applied arts being performed by self employed persons, an economic company or a craft collective. An individual person can perform a craft activity for which certain qualification repairements must be fulfilled and on the basis of craft permission and registration in the craft register [2]. The Slovenian craft with 52,109 craft enterprises and over 150,000 employees represents an important part of the Slovenian economy and the bulk of craft enterprises are SMEs, according to definition above. Special categories in the Slovenian craft are definitely craft enterprises without employees, which represent 55,4 % of all registered craft enterprises [3].

The total environmental impact of small firms is not known either at national or regional levels, but according to some surveys about 70 % of all industrial pollution is contributed by SMEs. The problem is not so much in individual firms, although in some cases there can be serious individual impacts on the local environments and communities, but rather in their combined total impact across sectors [4].

Over the years, worldwide concern for the environment is getting bigger attention. There are clear signals that wide industrial sectors are moving towards cleaner production processes, in order to respond, among other factors, to climate change imperatives. Industrial activities which cause an environmental pollution has been bounded to more comprehensive and effective environmental schemes or legislations (such as the IPPC Directive, the Directive on the Emission Trading Scheme), while SMEs often fall below the thresholds that trigger the application of instruments that concentrate on major individual sources of pollution. In cases, where environmental legislation is applicable to SMEs, they tend to presume that they are complying and, as a result, full compliance is often the result of external action after an inspection rather than an on-going process of checking that legal requirements are actually being met [5]. At the same time, SMEs often do not have the necessary legal and environmental expertise to cope with environmental legislation.

SMEs are responsible for the waste generated within their business; from when it is produced until they have transferred it to an authorized person.

SMEs are responsible for the waste generated within their business; from when it is produced until they have transferred it to an authorized person. The field of waste treatment within crafts is well regulated. Slovenian environmental policy has been based on the Waste Management Decree, and has been harmonized with the corresponding Euro-

pean Union's legislation. The amount of waste produced is usually lower than it is within the industry sector, but nevertheless the heterogeneous and diverse nature of the craft sector makes it difficult to generalize about the environmental impacts and strategies of the sector. Some of the empirical literature shows that the majority of SMEs have little awareness of the environmental impacts and how to manage them. Most of them (between 75 % and 90 % according to some surveys) think that their activities do not have any impact on the environment. Therefor, most of them have not introduced any practical measures to reduce their environmental impacts [6].

Actions are usually taken in order to avoid costs, but not at the expence of environmental protection.

SMEs usually do not have any employees dealing with environmental issues, so this remains the duty of the managing director or the business owner who also handles all other activities of the enterprise. It is hard for them to comply with environmental legislation. Actions are usually taken in order to avoid costs, but not at the expence of environmental protection [7]. Environmental planning, if any, in most cases is only of a short term and is applied as necessary. Sometimes companies have difficulties in understanding how the legislation affects them, which is accompanied by poor knowledge about their responsibilities according to the regulations; separating hazardous and special waste from others, false treatment and storing, keeping records and the awareness that these responsibilities have no time limit.

If SMEs are unaware of the environmental impacts of their own activities, and of the environmental legislation that applies to them, their activities could present an important threat for the environment. The environmental impacts of SMEs which are associated with low awareness and low legal compliance could also have the effect on increasing health and safety risks of workers. And at the end they can miss the chance to reap the economic benefits of the opportunities presented by better environmental management and eco-innovation [7].

The environmental impacts of SMEs which are associated with a low awareness and low legal compliance could also have the effect of increasing health and safety risks to workers.

The main hypothesis of this research work was that the majority of SMEs have little awareness of their environmental impact, particularly within the waste management operations, which makes them problematic in terms of compliance with environmental legislation. Through the research work we wanted to find out what is the level of knowledge about environmental requirements, waste and hazardous waste, waste minimization, and waste treatment in general between SMEs. We also wanted to find out how to provide possibilities that are not connected to high costs and investments for better waste treatment? By the help of questionnaires, interviews and on-site visits it has been endeavored to analyze the current situation, regarding how craft shops are dealing with waste and hazardous waste generated within business.

The study identifies some proposals for waste management improvements within SMEs starting with the prevention of waste generation in the first place. Also some other measures and tools to help SMEs to comply with the legislation and to improve their environmental attitude were identified.

The results consist of data collected through the analysis of returned questionnaires and of data collected from on-site interviews.

METHODS

The main objective of the study is to identify some proposals for waste management improvements within SMEs, starting with the prevention of waste generation, and other measures and tools to help SMEs to comply with the legislation and to improve their environmental attitude. The empirical part of presented work included a desktop research, which was in May 2008, continued with analyzing the questionnaires sent randomly using the data from the Craft Register [8], to 300 entrepreneurs who are members of the Chamber of Craft and Small Business of Slovenia from the section of maintenance of textiles and car services. The survey also included an on-site visit along with interviews conducted with some SMEs. The results consist of data collected through the analysis of returned questionnaires and of data collected from on-site interviews. The response rate was about 40 % and lower than had been anticipated, particularly from the car service section. There were 21 questions in the questionnaire; most of them were fixed-choice with two, three or more possible answers to select. In the introduction, it was asked about their core business and number of employees. It was continued with questions about quantities of waste and hazardous wastes produced per year, collecting and separating wastes at source, storing and recycling. It was foreseen that SMEs do not have full awareness of the legal requirements associated with their operation, so asking them about being aware of the demands of legislation. The questions following referred to specific waste treatment procedures which

Table 1:
Main themes of the interviews.

Waste and waste management within business: Actions to meet legislation and complying with legislation. Waste treatment within the business. Waste minimization, recovery, recycling. Removal by authorized person, disposal. Keeping the necessary documentation.
Awareness of environmental impact of the business: Is there any impact and what kind of? Responsibility towards the environment. Reducing environmental impacts. Environmental planning.
Environmental inspector visits: Supervisions within last five years. Measurements set down by the inspector. Improvements after the visit?
Need for further information and better advice: How, what? Guidance.
Motives to build up en EMS: Improvements regarding environmental issues. Willingness for on-going efforts. Short term plan to implement ISO 14001, EMASeasy. Expected benefits, obstacles.

are performed by each SME, such as: costs which appear yearly for hazardous and non hazardous waste treatment, having waste documentation as a waste management plan according to the Decree on waste management, who is involved in environmental issues, where do they usually get information and necessary knowledge and skills to comply with environmental issues, do they need additional education, do they have any controls and examinations by environmental inspectorate in their crafts units and finally, what measures were given, if any.

Interviews and on-site visits were meant as continuation work after the survey. Interviews were performed in May 2009, in selected companies within two businesses: car section, and textile maintenance 2009. The interviewing method was not based on a formal schedule of questions but the researchers had a list of selected themes for the discussion. On-site visits have followed the interviews and helped to better understand the situation and problems appearing within SMEs.

Main themes of the interviews with managing directors-owners as interviewee are listed in the Table 1.

RESULTS AND DISCUSION

The results of this study clearly showed that SMEs in general are very passive in environmental protection and that they primarily follow the development of tightening legislation. Actions taken are usually in order to avoid costs, not for sustainability or environmental protection. They often do not deal with environmental planning but if they do, planning is only for the short term. There are still some entrepreneurs who feel limited responsibility towards the environment. They often think they are small entrepreneurs and that their contribution towards environmental impacts is negligible. Sometimes, even if they were familiar with some options to improve their environmental attitude, there was no action taken because there was no willingness and positive aspiration to comply.

The results of this study clearly showed that SMEs in general are very passive in environmental protection and that they primarily follow the development of tightening legislation.

The results of the study showed that we are dealing with very small entrepreneurs (average number of employees is 3.1 with owners included), which are generating wastes and hazardous waste. A high percentage of those asked – 34 % – do not assort waste and dispose of the municipal waste, and from the answers which followed, it was clear that the knowledge about waste treatment is generally low. The Figure 1 shows different types of wastes which were stated as most common by entrepreneurs. It is obvious from the results that the surveyed entrepreneurs do not know enough about municipal wastes they listed only a few types of waste, in spite of the previous question in which many of respondents – 62 % – affirmed that they know about the municipal waste. They were asked which hazardous wastes have been generated within their business? Answers given by participants performing textile processing showed that they know their hazardous waste very well, while answers received from participants of the section of car services showed that they know only few of the hazardous wastes generated

It is obvious from the results that the surveyed entrepreneurs do not know enough about municipal wastes they listed only a few types of waste.

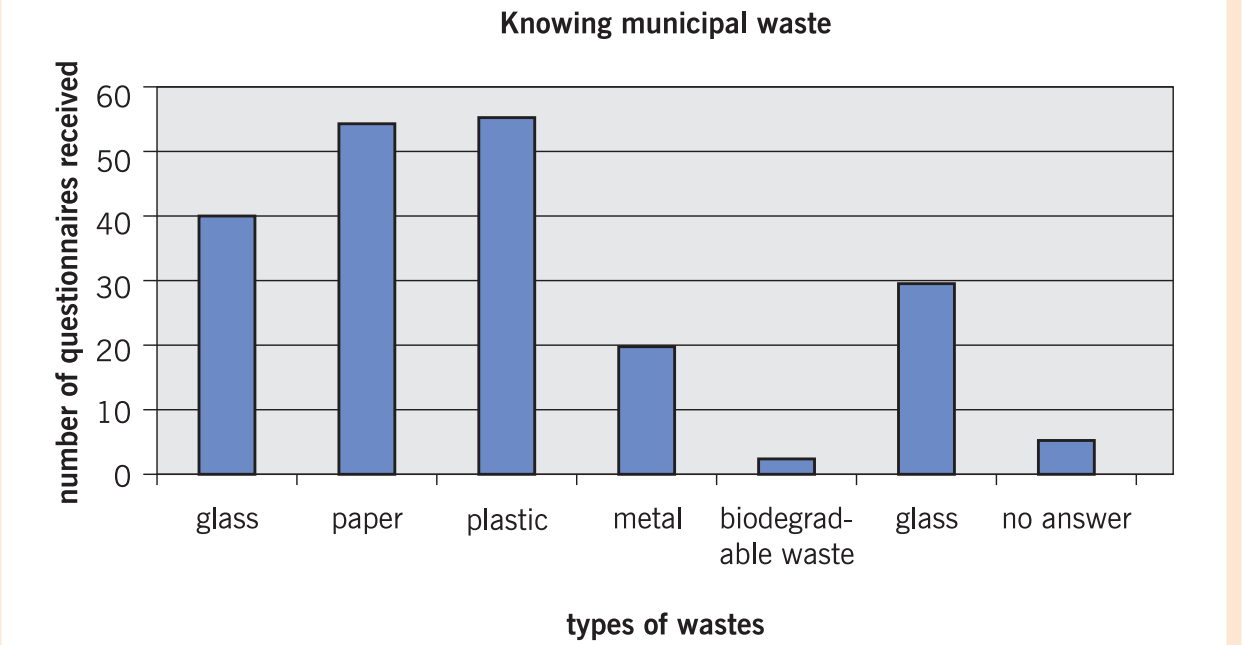
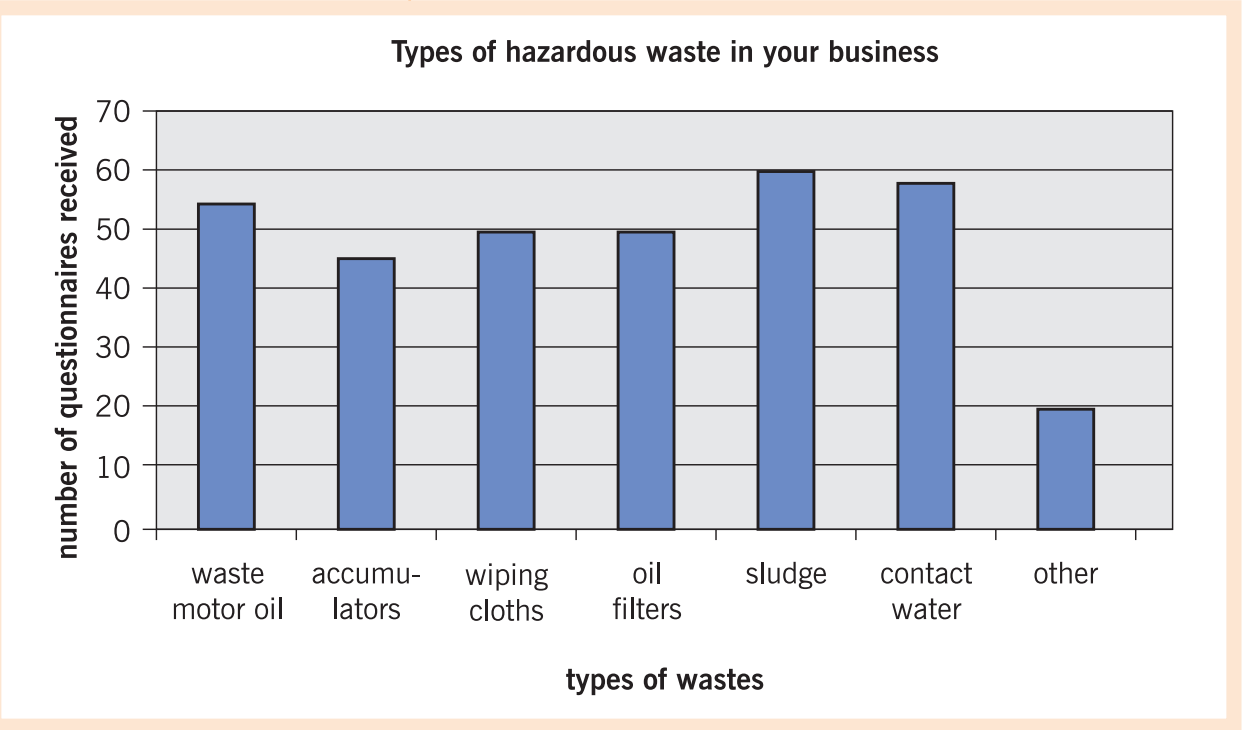


Figure 1.
Specify the municipal wastes which are being collected and disposed separately.

within their business (Figure 2). Mechanics are less aware of hazardous waste generated, even if there can be different types which can cause mismanagement with hazardous types of waste and pose a threat towards the environment and also to human health.

Only 10 % of the entrepreneurs surveyed generate less than 200 kg/a of hazardous waste and are consequently not obliged to prepare the waste management plan. But the following answers showed that more than 30 % of respondents thought they are not obliged to have the

Figure 2.
Which hazardous wastes are generated within your business?



plan. During the on-site visiting we noticed that some SMEs had a plan but had not cooperated when it was prepared. Unfortunately, the documentation prepared by an external company dealing with the environmental issues, usually stays in a drawer and the goals defined in documents are not followed.

Hazardous waste, according to the survey results, are collected separately in a high percentage, but there is almost no incentive to produce less hazardous waste. As was already foreseen, that none of the interviewed has the position of environmental officer. When we asked about the responsibilities for waste management, more than 70 % said that it is the duty of the owner of the company. This was an expected and important finding, since these positions are very demanding and usually they have no spare time for topics which are not directly connected to business.

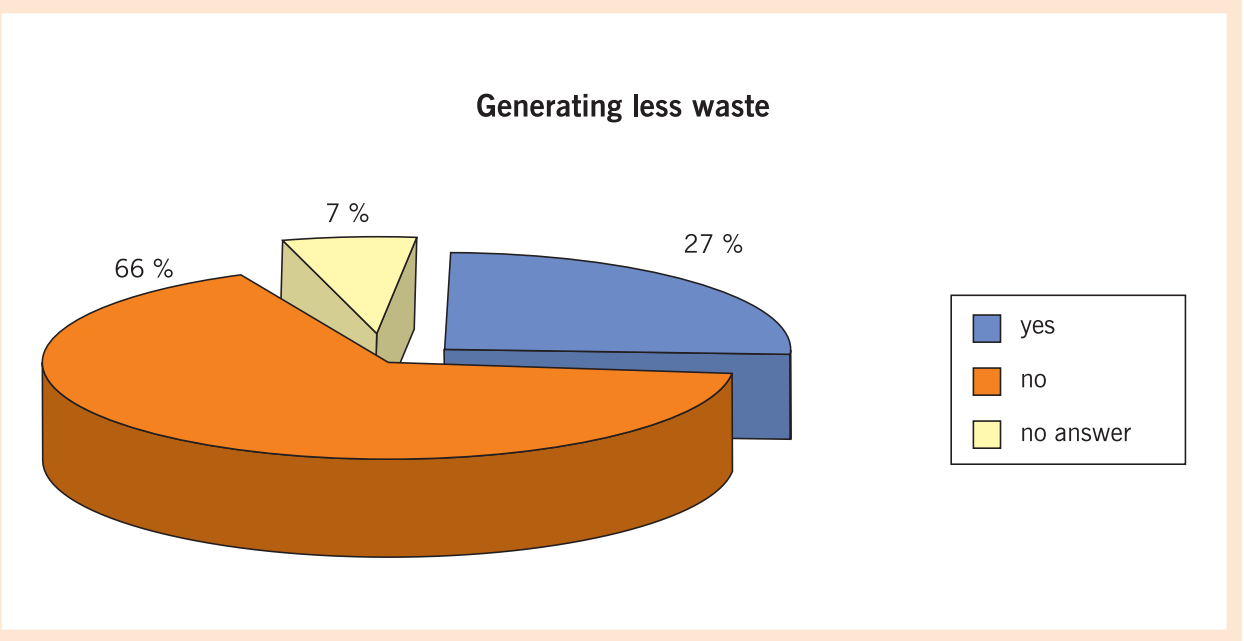
Hazardous waste, according to the survey results, are collected separately in a high percentage, but there is almost no incentive to produce less hazardous waste.

Almost 66 % of the participants (Figure 3) have not taken any measure to generate less waste which shows their passiveness towards environmental issues and little incentive to introduce waste reduction and minimization within the business.

More than 90 % of entrepreneurs stated that they do not recycle waste generated, which shows their passiveness towards the environment (Figure 4). The following questions about temporary waste collection showed that SMEs are using many wrong disposal places, which can be a threat either to human health and/or towards the environment.

More than 60 % of SMEs have not had supervision from the inspectorate within the last five years. It is known that many of the obligations from legislation are not followed and fulfilled until the inspectorate pays a visit and stipulate the measures. From the available data [9], it is clear that a great share of entrepreneurs seek help of the Chamber's consultants after receiving a visit from the inspectorate, stipulating the required measures. One of the entrepreneurs included in the survey,

Figure 3.
Do you try to generate less waste?



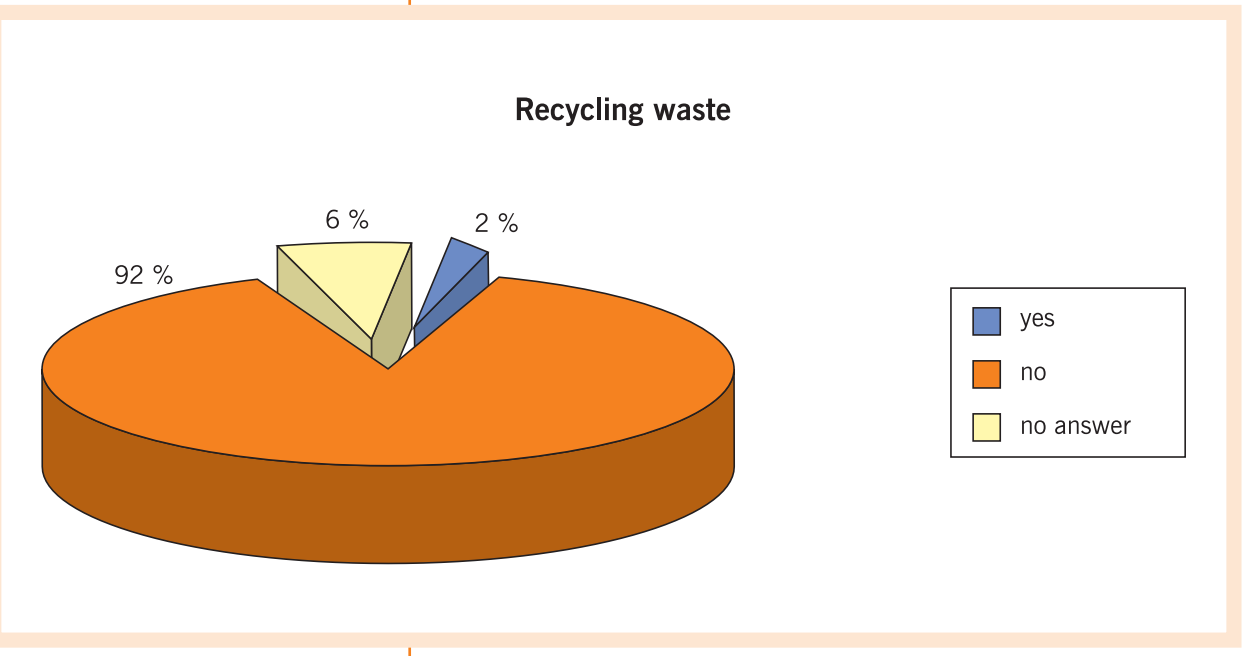


Figure 4.
Do you recycle the waste generated within your business?

that he was fined with a penalty since he was not separating wastes and hazardous waste according to the legislation. It is clear that actions such as inspections are insufficient to ensure compliance and permanent change in behavior among SMEs since the mismanagement in the same company within on-site visit was noticed.

They were also asked whether they have enough information for the proper waste treatment and almost 50 % of entrepreneurs included in the survey answered negatively answered that and are willing to acquire additional knowledge. From the results of the survey it was also noticed that SMEs are not familiar with simple on-site operations to reduce, re-use the waste, likewise they do not recycle wastes as they should. The need for better advice and additional educating was clearly expressed in the survey. Answers from the survey give good reasons for initiating the process of improving their waste management and environmental performance.

From the results of the survey it was also noticed that SMEs are not familiar with simple on-site operations to reduce, reuse the waste, likewise they do not recycle wastes as they should.

None of SMEs included in the on-site interviews has acceded to environmental planning or to take any proactive steps to improve their waste management. Most of the activities are taken in order to avoid costs, while hardly following the legislation. Many SMEs surveyed have not realized that separate and proper waste collection can increase the value of the waste; there are some opportunities for costs reduction, such as waste packaging or other forms of disposal.

CONCLUSIONS

The nature of wastes from SMEs is very heterogeneous; often there are several locations of waste origin which makes it difficult to generalize about the environmental impacts. That makes them a particularly problematic group in terms of complying with the legislation. SMEs, compared to larger firms, are unique in their heterogeneous nature and are fighting for their existence; occupying positions in most of the surveyed

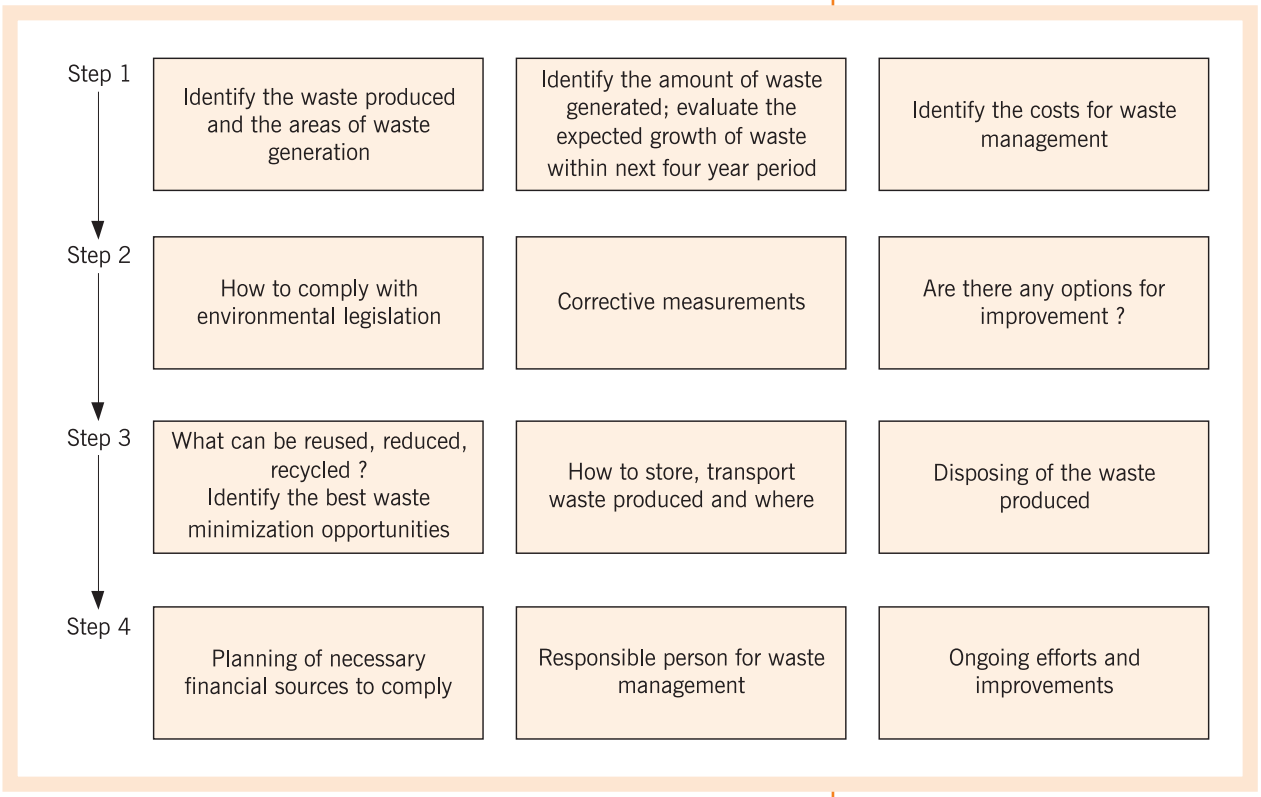
companies do not include environmental activities. That makes entrepreneurs vulnerable, and the lack of human and financial sources leads to low or non environmental planning, difficulties in understanding how legislation affects them, about their responsibilities determined by regulations, how to separate hazardous waste and special waste from others, where to put waste generated, how to collect temporary waste, to fill in and keep the documentation and the awareness that the environmental responsibilities of the polluter have no time limit.

Several methods to help SMEs to improve their environmental performance, particularly in the field of waste management were found. Many different options were taken into consideration to improve the waste treatment, to minimize the amount of waste and to reduce their hazardousness.

As a very important finding from the survey it was exposed and considered that SMEs suffer from a lack of legislation awareness and necessary know-how. If people who run the business are not complying with the legislation, it results in present mismanagement. All the key players should actively seek how to fill this gap successfully and to forward the necessary knowledge. Only SMEs who are aware of what the legislation demands are, can take actions to comply and thereafter to make a break from an old traditional approach and to adopt a proactive approach to environmental management of the business and consideration of environmental issues as an insignificant theme. A modern approach should be proactive, also with the environmental planning as stated in the Figure 5.

If people who run the business are not complying with the legislation, it results in present mismanagement. All the key players should actively seek how to fill this gap successfully and to forward the necessary knowledge.

Figure 5.
Schematic Approach to environmental planning.



Conclusions made after the survey offer a set of proposals for improving environmental performance:

– SMEs need additional knowledge and know-how to improve their environmental attitude; this can be achieved through preparing on-going seminars, round tables, branch meetings with environmental themes discussed, etc.

– Concrete solutions through technical and organizational measures have to be prepared: how properly treat the waste generated, the possibilities for waste minimization on-site, how to reduce the amounts of hazardous waste, how to reuse, recycle and recover, and follow instructions for proper temporary waste collection. Waste recycling operations that would take place within SMEs are not appropriate solution, when taking into consideration the recycling warranted from the point of economy (costs would be significant where the amounts of wastes are small) and environmental protection. But what entrepreneurs can successfully do is to take care that wastes are collected and separated in a way that further *procedures* such as recycling are possible. It also has to be stressed that every employee has to be educated and motivated to take care. Since these are SMEs with only few employees, the proposals stated will only work successfully if everyone in the company is familiar with and engaged in the process of proper waste treatment, not only the managing director. There were no companies in the site visiting which would have a staff member responsible for at environmental performance issues. This fact probably would not change in the near future (unless legislation changes) from the point of costs. The duty of owners is to change consideration of the environment as a peripheral issue and to give a good example to everyone else engaged in business. This form of help should be assured to as many SMEs as possible. For this purpose a unique web site will be completed (by the Chamber of craft and small business of Slovenia) containing all important data on environmental issues by for different businesses and topics, tackling the entrepreneurs starting a business or already working.

Waste recycling operations that would take place within SMEs are not appropriate solution, when taking into consideration the recycling warranted from the point of economy and environmental protection.

– Assistance for SMEs has to be assured and strengthened through their supporting organizations such as chambers, nongovernmental organizations, waste carriers in order to offer better advice, and tailor made advice from knowing the uniqueness of each business.

– The role of government should not only be in making regulations but also in encouraging SMEs to improve their environmental attitude and waste management by participating in and implementing voluntary programmes such as cleaner production, ISO 14001, EMAS (Eco-Management and Audit Scheme), EMASeasy (a lean and standardized methodology for small and micro businesses, based on the Eco-mapping© concept) [10] and eco-label. By all expressed measurements, government has the goal to cut the red tape and to simplify the existing legislation where possible. Government is obliged to stimulate the adoption of voluntary programmes with fairer fees and

to enable more support, but there arises the question of whether SMEs are ready to take such a big step. Probably it is too soon to think about that at least for the smallest companies which have been now fighting to overcome the crucial gap in know-how and knowledge. But yet, the EMASeasy is an excellent tool also in developing environmental understanding-what many entrepreneurs need to be able to assure environmental compliance.

In order to achieve successful waste treatment and to ensure lasting benefits, merely complying with the regulations as a traditional way of approach towards the environment is not enough. We have to develop new approach based on co-ordination of certain key players by changing the companies' culture towards a more environmentally responsible, to engage actively in improving their environmental performance and their ethos, whatever their size or business is.

We have to develop new approach based on co-ordination of certain key players by changing the companies' culture towards a more environmentally responsible, to engage actively in improving their environmental performance and their ethos, whatever their size or business is.

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Pregledni strokovni članek

Gensko spremenjeni organizmi in tveganja za zdravje

Health Risk and Genetically Modified Organisms

Sebastjan **FILIP**[1], Rok **FINK**[2*]

POVZETEK

Že od nastanka prvih civilizacij človek spreminja podobo narave s križanjem in drugimi kmetijskimi pristopi. Vendar je šele razvoj genskega inženirstva omogočil izmenjavo genskega materiala med vrstami, ki med seboj niso v sorodu. Tako nastale nove rastlinske in živalske vrste so sprožile številne etične, okoljske in tudi zdravstvene pomisleke. Čeprav je namen gensko spremenjenih organizmov (GSO) doseči večji donos, odpornost proti škodljivcem in boleznimi, imajo GSO tudi vplive na zdravje ljudi in živali. Znani so številni primeri transgenega prenosa genov, ki določajo izražanje toksinov, alergenov ali povzročajo rezistenco mikroorganizmov. Takšni primeri so na stopnji poskusov povzročili pogine laboratorijskih živali. Številne raziskave poročajo o pojavu resistence mikroorganizmov, antinutrientih tiaminaze in ω-6 maščobnih kislin, poginu glodavcev, ki so bili hranjeni z transgeno koruzo in podobno. Vendar pa ima implementacija gensko spremenjenih organizmov tudi pozitivne učinke, saj je uvedba zlatega riža, ki je bogat vir β-karotena, rešila številna življenja otrok v Afriki in Aziji. Poleg tega so transgene rastline bolj odporne na škodljivce in podnebne spremembe, ki postajajo vsakdanjost današnjih generacij. V prihodnosti je potrebno raziskave usmeriti v proučevanje dolgoročnih vplivov na zdravje, predvsem v smislu potencialnega vpliva na človeški genom. Med tem pa zagotoviti ustrezno zakonodajo in analizo tveganja vsakega potencialnega GSO, po principu previdnostnega načela bistvenega pomena, še preden pride takšen organizem v prehransko verigo.

KLJUČNE BESEDE:

gensko spremenjeni organizmi, zdravje, tveganje, hrana, toksičnost, alergenost.

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ABSTRACT
Since the first civilisations humans began to modify nature using breeding or other agricultural techniques. However, it is only the development of genetic engineering that enabled the exchange of genetic material between species that are not related. Although the goal of genetically modified organisms is to achieve higher yields, resistance to pests and diseases, there are also GMOs effects on human and animal health. Several studies report the *occurrence* of bacterial resistance, thiaminase and ω-6 fatty acids, the death of rodents that were fed with transgenic corn. However, the implementation of genetically modified organisms has also positive impacts, since the introduction of golden rice, which is a rich source of β-carotene, is saving lives of children in Africa and Asia. In addition, the transgenic plants are more resistant to pests and climate change and are becoming the norm today's generations. In future research should be directed to the study of long-term effects on health especially in term of the potential impacts on human genome. Meanwhile the relevant legislation and risk assessment of single GMO on the precautionary principle is essential before such organisms are released in the food chain.

KEY WORDS:
Genetically modified organism, Health, Risk, Food, Toxicity, Allergenic.

UVOD

Človeštvo si že od nastanka prvih civilizacij podreja in spreminja okolje v katerem živi [1]. Vendar se je ta želja po nenehnem obvladovanju narave razvijala od enostavnega zavetja pred neugodnimi vremenskimi vplivi preko industrijske revolucije in raziskovanja vesolja, vse do posega v najmanjše, a najbolj intimne koščke življenja – v genski zapis žive celice. GSO (gensko spremenjeni organizem) je organizem z izjemo človeškega, pri katerem je genski material spremenjen na način, ki se ne pojavlja v naravi z razmnoževanjem ali naravno rekombinacijo [2]. GSO so že vstopili v prehrambno verigo v večini predelov sveta. Veliko mikroorganizmov, predvsem pa bakterije in glive so bile spremenjene z namenom povečanja produkcije proteinov, aminokislin in drugih komercialnih sestavin ali surovin. Kot vse nove tehnologije, tudi tehnologija genskega inženirstva sproža določena vprašanja in dileme, ki se nanašajo na varnost okolja in človeka [3]. Zato je namen prispevka pregledati in kritično analizirati rezultate raziskav o vplivu GSO na zdravje ljudi. Pri tem smo avtorji oblikovali dve hipotezi:

Namen prispevka je pregledati in kritično analizirati rezultate raziskav o vplivu GSO na zdravje ljudi.

- **H1:** Rezultati raziskav na področju vpliva GSO na zdravje opredeljujejo tako pozitivne kot negativne učinke uporabe GSO.
- **H2:** Raziskava o vplivu GSO na zdravje ljudi določajo le kratkoročne vplive kot so akutna toksičnost in alergogenost, ne definirajo pa dolgoročnih in kroničnih učinkov na zdravje pri uživanju GSO.

ZGODOVINSKI PREGLED GENSKEGA INŽENIRSTVA

Skoraj 8.000 let pred našim štetjem je človek začel gojiti rastline s tem, ko je divje vrste, kot so žito, fižol, leča in grah križal s sorodnimi vrstami z namenom boljšega donosa ali druge kmetijske prednosti kot so odpornost na sušo, insekte ali bolezni. Tako je danes žito razširjeno vse od

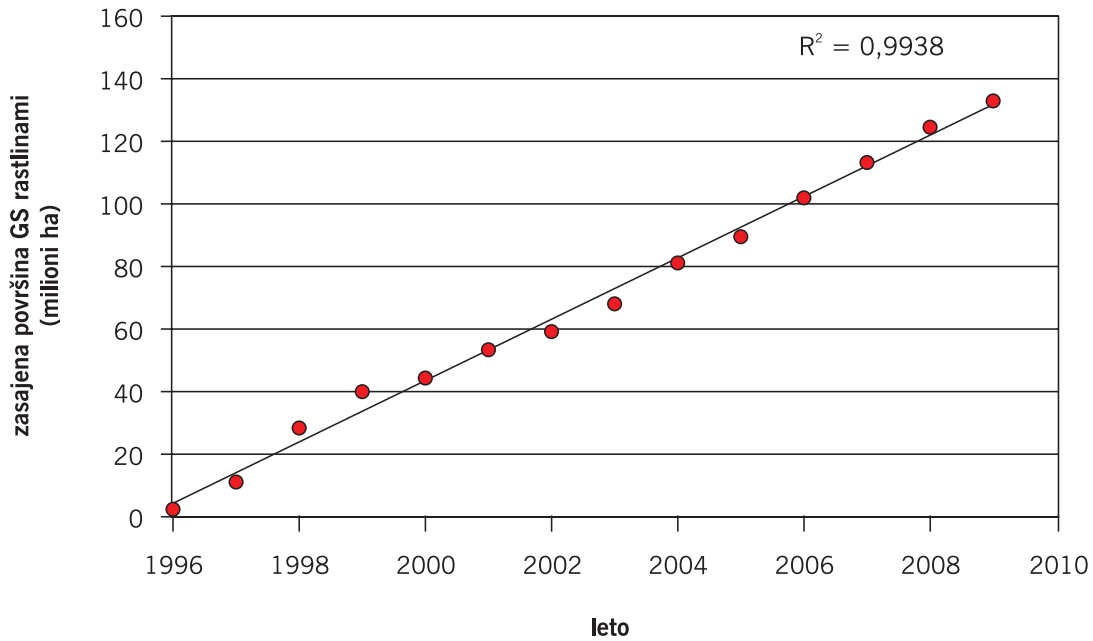
Gensko inženirstvo tako vključuje postopke s katerimi namerno spreminjamo genski material rastlin ali živali, kar se odraža v njihovih novih lastnostih.

Skandinavije in Argentine do visokogorja, tropskega in subtropskega podnebja [4]. Križanja in podobni postopki so privedli do sprememb genskega materiala teh rastlin do takšne mere, da rastline niso bile več sposobne preživeti v divjini oz. v njihovem naravnem okolju [5]. V tej dolgi zgodovini so se kmetje pri križanju rastlin zanašali le na izbiro primernih vrst, variacij in kultur. Šele z Mendeljevim odkritjem genetske zakonitosti dedovanja fenotipskih lastnosti je koncept križanja rastlin postal dostopen vsem [6]. Vendar pa je bil takrat možen prenos genskega materiala le med sorodnimi vrstami. Pred približno petdesetimi leti pa je gensko inženirstvo omogočilo prenos specifičnih genov ali njihovih skupin med vrstami, ki med seboj niso v sorodu. Takšni postopki so omogočili prenos genskega materiala na primer bakterij v rastline, kar s klasični metodami križanja ni mogoče [7].

Gensko inženirstvo tako vključuje postopke s katerimi namerno spreminjamo genski material rastlin ali živali, kar se odraža v njihovih novih lastnostih. Le te se lahko odražajo kot odpornost na herbicide, viruse, insekte in bolezni [8, 9]. Sposobnost manipulacije genskega materiala in prenosa iz ene vrste v drugo zaradi ekonomskih razlogov je osnovni namen biotehnološke industrije [10]. Ena izmed prvih transgenih rastlin je bil krompir Lenape, ki je vseboval visoko vrednost suhe snovi in je bil kot takšen primeren za proizvodnjo čipsa. Vendar je bi že dve leti po začetku uporabe opažen pojav toksina solanina in je bil zato umaknjen iz prodajnih polic v Združenih državah Amerike [11]. Leta 1979 so na Univerzi v Cornellu v New Yorku proučevali sintetični rastni hormon pridobljen s pomočjo biotehnologije, ki je pri kravah molznicah povečal produkcijo mleka. Okoli leta 1990 so GS organizmi postali komercialno dostopni skoraj po vsem razvitem svetu in njihova uporaba se iz leta v leto povečuje (slika 1). V začetku devetdesetih let prejšnjega stoletja so številne ugledne ameriške organizacije kot so Ameriško medicinsko

Slika 1.
Zasajene površine v milionih hektarjih z GS rastlinami v obdobju 1996-2009 [12].

Figure 1.
GM planted areas in million hectares from 1996 to 2009 [12].



združenje (American Medical Association), Ameriški nacionalni inštitut za zdravje (National Institute for Health), Uprava za prehrano in zdravila (Food and Drug Administration) in številni drugi, podali mnenje, da so transgeni organizmi prav tako varni kot konvencionalni [10]. Že sredi devetdesetih let pa je uporaba genskega inženirstva za kloniranje celic sprožila nov val dvomov in upora proti GS organizmom. Tako so številne vladne in nevladne organizacije zahtevale celovito analizo vplivov GS organizmov na zdravje ljudi in okolje.

Transgene rastline imajo v primerjavi s konvencionalnimi večji donos in so praviloma bolj obstojne na neugodne vremenske pogoje in škodljivce.

PREDNOSTI IN SLABOSTI GS ORGANIZMOV

Tehnologije genskega inženirstva imajo prednosti in slabosti (tabela 1). Transgene rastline imajo v primerjavi s konvencionalnimi večji donos in so praviloma bolj obstojne na neugodne vremenske pogoje in škodljivce. Pričakovane globalne spremembe bodo močno prizadele kmetijski sektor z znižanjem produkcije nekaterih rastlin [13]. Medtem ko kritiki izražajo dvome v varnost GS živil, navajajo primere alergičnih reakcij in toksičnosti. Philips [14] navaja, da genskega materiala ni mogoče v vseh primerih transportirati do tarčnih celic oziroma se lahko genski material veže na neželeno mesto v DNA verigi. To lahko povzroči aktivacijo sosednjih genov, ali zavre izražanje drugih, kar lahko posledično povzroči neželeno mutacijo, ki ima za posledico pojav toksina.

Tabela 1.
Prednosti in slabosti GS organizmov.

Table 1.
Advantages and disadvantages of GM organisms.

Prednosti	**Reference**
Večji hektarski donos rastlin	[10, 15-18]
Nižja cena končnega produkta	[16]
Večja odpornost na sušo	[19]
Slabosti	**Reference**
Manjša kvaliteta živil	[14, 20]
Odpornost na antibiotike	[14, 21, 22]
Potencialna toksičnost	[14, 22]
Potencialno tvorjenje toksinov	[22-24]
Nenameren prenos genov z GS rastline na konvencionalno rastlino	[25-28]
Potencialno tvorjenje novih virusov	[14, 29-31]

ZAKONODAJA

Leta 1996 je prva pošiljka GS koruze pripotovala iz Amerike v Evropo in tako sprožila številne okoljske in potrošniške organizacije, ki so nasprotovale novi tehnologiji ter pritegnile pozornost medijev. Največja skrb je bila potencialna nevarnost nenadzorovanega širjenja GSO in negativnega vpliva na okolje ter zdravje ljudi. Številna gibanja nevladnih organizacij so sprožila odlog o avtorizaciji GSO, ki je postal učinkovit šele leta 1999, ko je Evropska skupnost revidirala direktivo iz leta 1990 (EU Direktiva 90/220/EEC) o okoljskem sproščanju GSO [32]. Takšen ukrep je

Danes morajo vsa nova živila (kamor spadajo tudi GS živila) skozi postopek analize tveganja.

omogočil blokado vseh nadaljnjih avtorizacij GSO in njihovo sproščanje v okolje. Od konca devetdesetih let se neprestano oblikuje in dopolnjuje zakonodaja na področju GSO, ki ima predvsem dva cilja; zaščititi zdravje ljudi, živali in okolja pred sproščanjem GSO in uživanjem živil, ki vsebujejo GSO. Zagotoviti pretok zdravih in varnih GS živil na ozemlju EU [33]. Danes morajo vsa nova živila (kamor spadajo tudi GS živila) skozi postopek analize tveganja, ki zajema naslednje korake:

- ***Identifikacija tveganja*** pomeni opredelitev ali je neka snov, kot npr. sestavina živila, vzročno povezana z določenimi vplivi na zdravje. Tveganje se določa eksperimentalno v kontrolnih toksikoloških študijah z znano dozo izpostavljenosti toksičnemu agensu. V praksi se pri statističnih ugotovitvah navaja največji tolerantni odmerek; to je največji odmerek, ki ga je moč zaznati v večini študij z laboratorijskimi živalmi.
- ***Odmerek – odziv*** pomeni določitev povezave med obsegom izpostavljenosti in verjetnostjo pojava škodljivega vpliva. Veliko snovi ima škodljive učinke šele pri večjih odmerkih, zato bi lahko brez ocene odmerka – odziva prišli do napačne ugotovitve.
- ***Ocena izpostavljenosti*** je ocena trajanja izpostavljenosti škodljivi snovi pod določenimi okoliščinami.

Tveganje je ocena prvih treh faktorjev in je pogosto podano kot kvantitativna ocena verjetnosti škodljivega učinka. Tveganje je verjetnost, da bo ravnanje z GSO posredno ali neposredno, takoj ali kasneje ali dolgoročno kumulativno škodljivo vplivalo na okolje ali zdravje ljudi.

GS živila morajo pred vsako sprostitvijo na trg pridobiti dovoljenje, ki dokazuje, da ni pričakovati škodljivih posledic na zdravje ljudi.

Namen analize tveganja je zagotoviti ustrezne informacije, ki omogočajo v postopku odločanja varovanje zdravja ljudi pred nesprejemljivimi tveganji [34,35]. Tako analiza tveganja predstavlja osnovo za zagotavljanje varnosti GSO.

VARNOST GSO

Varna hrana pomeni zagotovilo, da živilo ne bo povzročilo škode potrošniku, če je pripravljeno in zaužito v skladu z namenom uporabe. GS živila morajo pred vsako sprostitvijo na trg pridobiti dovoljenje, ki dokazuje, da ni pričakovati škodljivih posledic na zdravje ljudi [36]. Tveganja za zdravje ljudi, ki so povezana z GS živili posegajo predvsem na štiri področja zdravja:

- ***Sprememba prehranske vrednosti:*** genski material tujega organizma lahko vpliva na prehransko vrednost živila, saj lahko spremenjen genski zapis poviša ali zniža vrednost posameznega hranila. Na področju vpliva GSO na prehransko vrednost živil je malo raziskav, saj ni natančno znana biodostopnost GS živil, interakcija med genom, hranilom in njihov metabolizem.
- ***Rezistenca na antibiotike:*** v genskem inženirstvu se geni, ki vsebujejo tudi rezistenco na antibiotike pogosto uporabljajo v tarčnem organizmu. Splošna razširitev takšnih genov v populaciji rastlin in živali lahko pripomore k javnozdravstvenemu problemu rezistence mikroorganizmov na antibiotike.

- ***Potencialna toksičnost:*** genske modifikacije lahko namerno ali nenamerno sprožijo izražanje genov v rastlinah in živalih, ki sprožijo nastajanje naravnih toksinov. Takšni primeri so inhibitorji proteaz v leguminozah, cianogeni v fižolu in Juki (*Yucca* sp.) ter tiramin v bananah.
- ***Potencialna alergenost*:** z gensko modifikacijo lahko prenesemo tudi alergene lastnosti donorja v rastlinskega ali živalskega prejemnika. Prav gensko inženirstvo kot donorje najpogosteje uporablja mikroorganizme, katerih alergenost ni znana ali analizirana. Podjetje *Pioneer Hi-bred International* je vgradilo genski material brazilskega oreščka (*Bertholletia excelsa*) v sojo, kar je sprožilo številne alergične reakcije med potrošniki, ki za zaužili GS sojo in so bili alergični na brazilski orešček [24].

Ena od glavnih skrbi, ki jih prinašajo GS živila, je potencialna nevarnost sproščanja intrinzičnih antinutrientov in toksinov. V ribah napihovalkah je prisoten nevrotoksičen alkaloid terodotoksin, zato obstaja nevarnost transgenega prenosa genskega materiala na druge vodne organizme [37]. Drugi znan primer prenosa je encim tiaminaza, ki je pomemben antinutrient, saj razgrajuje vitamin B1. Iz azijskih držav so poročali o poginu psov in mačk, ki so bili hranjeni s transgenimi ribami z visoko koncentracijo tiaminaze [38]. Med tem ko je bil pri prašičih, ki so bili tretirani s GS rastnimi hormoni, opažen nižji delež maščobnega tkiva. Takšno meso je vsebovalo manj nasičenih in več nenasičenih maščobnih kislin kot kontrolni prašiči [37]. Guillen s sodelavci [39] je analiziral vpliv rastnega hormona ribe tilapije (*Tilapia* sp.) na makakije (*Macaca* sp.). Primati so bili izpostavljeni intravenoznemu injiciranju 1,0 μg/kg rastnega hormona v obdobju 30 dni. Riba tilapija vsebuje visok delež omega-6 maščobnih kislin, predvsem na račun arahidonske kisline in je bil med makakiji zaznan visok delež ateroskleroze glede na kontrolno skupino. Medtem Maclean [40] navaja, da GS ribe ne predstavljajo večjega tveganja za zdravje ljudi kot gojene ribe, ki so izpostavljene antibiotikom, cepivom, adjuvansom in aditivom kot je na primer karoten v ribjih krmilih [40]. Težavo lahko predstavljajo transgene ribe, ki imajo gen, ki kodira tvorbo toksinov iz drugih živali. Če ima riba kod za tvorbo toksinov, potem takšna riba ni primerna za prehrano ljudi ali živali [40].

Ena od glavnih skrbi, ki jih prinašajo GS živila, je potencialna nevarnost sproščanja intrinzičnih antinutrientov in toksinov.

Iz področja rastlinske genetike je medijsko zelo odmeven primer transgene koruze MON 863, ki je vsebovala gen za odpornost proti koreninski gnilobi. GS koruza je vsebovala insekticid Cry3Bb1, vendar test toksičnost pri sesalcih ni bil opravljen. Prav tako pri insektih tarčni receptorji vezave toksina niso bili točno poznani [41]. V raziskavi so testne podgane hranili s transgeno koruzo MON 863 v obdobju 90 dni. Rezultati so pokazali statistično značilno ($p < 0,01$) povezavo med znižanjem telesne teže podgan, hepatorenalne toksičnosti, povišanjem trigliceridov, ter zmajšano izločanje natrija in fosforja v primerjavi z kontrolno skupino podgan, ki je bila hranjena le z ne GS koruzo [42].

Bacillus thuringiensis je gram pozitivna bakterija s sposobnostjo proizvodnje insekticidih proteinov, ki učinkujejo proti Lepidopteram (*Lepidoptera*) in drugim insektom [43]. Vendar lahko nekateri sevi bakterije

Poznani so posamezni primeri, ko so živali poginule zaradi uživanja rastlin, katerim ki jim je bil vnesen gen omenjenega bacilusa.

Omenjena modifikacija riža naj bi predstavljala enega izmed načinov, kako se boriti proti pomanjkanju vitamina A.

Veliko otrok s pomanjkanjem vitamina A trpi tudi za podhranjenostjo in infekcijami, ki ovirajo absorbcijo beta karotena in njegove pretvorbe v vitamin A.

proizvajajo tudi beta eksotoksine, ki pa so toksični za vretenčarje in nevretenčarje [44]. Poznani so posamezni primeri, ko so živali poginule zaradi uživanja rastlin, katerim jim je bil vnesen gen omenjenega bacilusa. Cohen [45] pa poroča, da je v petih letih od uporabe transgenih rastlin, že več deset vrst insektov razvilo odpornost na toksin *Bacillus thuringiensis*.

Zlati riž je GS konvencionalen riž (Oryza sativa), ki proizvaja β-karoten v endosperminjih. Beta karoten je namreč prekurzor vitamina A [46,47]. Pridelava le-tega s pomočjo genskega inženiringa je bil edini način, saj plazme riža, ki bi bila sposobna sintetizirati karotene v endospermih, namreč ni. Endosperm zlatega riža je rumen (zlat) zaradi kopičenja β-karotena in ksantofilov. Omenjena modifikacija riža naj bi predstavljala enega izmed načinov, kako se boriti proti pomanjkanju vitamina A, ki je globalni zdravstveni problem in ga je čutiti predvsem v manj razvitih državah Azije pa tudi Afrike. Le-tega naj bi revno mestno in podeželsko prebivalstvo, predvsem tisto, ki živi v oddaljenih krajih, dobila s pomočjo kmetijstva in lokalne prodaje [48]. Vendar ima efekt zlatega riža zemljepisne omejitve. Riž je v Aziji zelo pomemben, medtem ko v predsaharskem delu Afrike temu ni tako [49]. Pojavila so se tudi vprašanja o biološki dostopnosti β-karotena v zlatem rižu. Nekateri znanstveniki trdijo, da ne bo pomagal pri boju s pomanjkanjem vitamina A, ker naj bi več faktorjev preprečilo efektivno izrabo β-karotena v zlatem rižu. Prebava, absorbcija in transport β-karotena zahteva proteine in skladišča maščob. Veliko otrok s pomanjkanjem vitamina A trpi tudi za podhranjenostjo in infekcijami, ki ovirajo absorbcijo beta karotena in njegove pretvorbe v vitamin A [50,51].

ZAKLJUČEK

GSO in hrana so na tržišču dostopni le nekaj desetletij, študij, ki bi vrednotile dolgoročni vpliv le teh na zdravje ljudi ni.

Zato ni mogoče samo enostransko določiti, ali imajo GSO pozitiven ali negativen vpliv na zdravje.

Pri pregledu in analizi raziskav lahko opazimo, da vse študije temeljijo na kratkoročnih vplivih na zdravje ljudi in ostalih organizmov.

Problematika GSO se je razširila iz individualne skrbi laboratorijev na globalno raven celotnega prebivalstva. Še več, problem ni le strokovne narave, kajti z vpletanjem človeškega življenja se pojavijo zdravstvene in druge razsežnosti in tematike, ki jih danes še ne poznamo, oziroma se ta področja še raziskujejo. Dileme o varnosti GSO v živilih so v zadnjem desetletju pritegnile mnogo pozornosti in sprožile številne ukrepe na področju zakonodaje. GSO in hrana so na tržišču dostopni le nekaj desetletij, študij, ki bi vrednotile dolgoročni vpliv le teh na zdravje ljudi ni. Številne raziskave o GSO opredeljujejo tako prednosti uvajanja GSO, kot njihova tveganja. Zato ni mogoče samo enostransko določiti, ali imajo GSO pozitiven ali negativen vpliv na zdravje. Raziskave poročajo o sproščanju številnih intrinzičnih antinutrientov in toksinov iz transgenih organizmov in znani so primeri pogina živali, ki so se hranile s takšnimi organizmi. Vendar lahko zasledimo tudi uspešne primere uporabe GSO kot je uporaba "zlatega riža" v tretjem svetu, večji donos in manjša občutljivost transgenih rastlin na podnebne spremembe. Zato lahko prvo hipotezo potrdimo. Pri pregledu in analizi raziskav lahko opazimo, da vse študije temeljijo na kratkoročnih vplivih na zdravje ljudi in ostalih organizmov. Poznamo primere cianogenih spojin v fižolu, juki in bananah, alergogenost transgene soje, ki vsebuje gen brazilskega oreščka,

encima tiaminaze v ribah in številne druge. Vendar raziskave ne poročajo o potencialnih dolgoročnih vplivih na zdravje ljudi. Študije, ki proučujejo dolgoročne vplive, so dolgotrajne in ker so GSO relativno mlada tematika, bo potrebno na rezultate nekaj časa počakati. Študije, ki so v teku še nekaj časa ne bodo zaključene, saj proučevanje dolgoročnih vplivov zahteva čas. Potrebno je uvajanje novih tehnologij in slediti principu previdnostnega načela, kjer mora biti vsak posamezni primer implementacije spremljan in analiziran, preden transgen organizem preide v prehransko verigo človeka ali živali. Danes ni več vprašanje ali bodo GSO igrali pomembno vlogo v prihodnosti, vendar je v ospredu vprašanja kakšna bodo tveganja za zdravje ljudi in okolje. V prihodnosti se morajo raziskave usmeriti v proučevanje dolgoročnega vpliva GSO na zdravje predvsem z vidika toksičnosti, sinergizma in vpliva na genski kod človeka.

Potrebno je uvajanje novih tehnologij in slediti principu previdnostnega načela, kjer mora biti vsak posamezni primer implementacije spremljan in analiziran, preden transgen organizem preide v prehransko verigo človeka ali živali.

V prihodnosti se morajo raziskave usmeriti v proučevanje dolgoročnega vpliva GSO na zdravje predvsem z vidika toksičnosti, sinergizma in vpliva na genski kod človeka.

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INSTRUCTIONS FOR AUTHORS

Scope

Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research presents broad interdisciplinary information on the practice and status of research in environmental, food and occupational hygiene, epidemiology, the environmental engineering science, systems engineering, and sanitation. Papers focus on design, development of health engineering methods, management, governmental policies, and societal impacts of drink water, wastewater collection and treatment; the fate and transport of contaminants on watersheds, in surface waters, in groundwater, in the soil, and in the atmosphere; environmental biology, microbiology, chemistry, fluid mechanics, and physical processes that control natural concentrations and dispersion of wastes in the air, water, and soil; non-point source pollution on water-sheds, in streams, in groundwater, in lakes, and in estuaries and coastal areas; treatment, management, and control of hazardous wastes; control and monitoring of air pollution and acid deposition; air-shed management; and design and management of solid waste professional obligations facilities; food technology, management of food quality and food safety. A balanced contribution from consultants, sanitary engineers, and researchers is sought on engineering solutions and responsibilities.

Submission of Articles

All manuscript for Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research should be submitted to:

Inštitut za sanitarno inženirstvo
Institute of Occupational, Food and Environmental Hygiene
Zaloška cesta 155
SI - 1000 Ljubljana
Slovenia
E-mail: info@institut-isi.si.

Your article will be submitted to the review process. All correspondence, including notification of the Editor's decision and request for revision, takes place by e-mail.

Submission of a paper implies that it has not been published previously, that it is not under consideration for publication elsewhere, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written permission of the publisher. Authors are solely responsible for the factual accuracy of their papers.

Types of Contributions

Original research articles: Research articles of 6,000-10,000 words (10 – 18 manuscript pages) in length, with tables, illustrations and references, in which hypotheses are tested and results. Research articles report on significant and innovative achievements, approaches and should exhibit a high level of originality.

Technical articles: Technical articles at least of 6,000 words (to 12 manuscript pages) in length, with tables, illustrations and references. Technical articles should re-

port on significant and innovative achievements of an already described innovation, experiences, state of the art technologies and know-how that are not based on new experiments and research.

Governmental initiatives: Reports on new or existing policies, governmental initiatives and programmes, have up to 2,000 words.

Educational Initiatives: Reports on research activities, education and training and new courses/approaches at academic institutions, training centres, initiatives of transfering knowledge, e-learning etc. The report should have 1.000 - 3.000 words (2 - 3 manuscript pages).

Conference reports: Reports on domestic and international conferences in particular interest to the readers of the Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research. The length of the article should reach up to 2.000 words.

Book reviews, Software reviews: Reviews on new books and software relevant to the scope of the Journal of Sanitary Engineering, of approximately 500 – 1,000 words.

Calendar of Events: Forthcoming meetings, workshops and conferences of relevance to scope of the Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research. The note should provide information on the date, title and venue, contact addresses for further contacts and the web page information if available.

Manuscript preparation

Cover letter: Cover letter should contain:

1. Title of the paper (without any abbreviations).
2. Full name(s) of the author(s).
3. Affiliation and addresses of the author(s).
4. Mailing address of the corresponding author (address, phone and fax number, e-mail).
5. A statement that the article is original, the manuscript or any part of it (except abstract) has not been sent to any other publisher or it is not in consideration for publication anywhere in any language.
6. A statement, that all authors read the article and agree with the content.
7. Written permission of the publisher for the use of tables, figures and any other part of the material in original form used in the article.
8. A statement of the ethnical principals used during the experiments with animals (if any).

A copy of the cover letter must be signed by the corresponding author and sent to the editor by regular mail.

General: Manuscript must be prepared with Word for Windows, double-spaced with wide margin 25 mm. 12 pt Times New Roman font is recommended. Correct spelling and grammar are the sole responsibility of the author(s). Articles should be written in a concise and succinct manner. Research papers should have the following structure: 1. Title, 2. Authors and affiliations, 3. Abstract (max. 200 words), 4. Keywords (max. 6 words), 5. Introduction, 6. Methods, 7. Results and discussion, 8. Acknowledgements (if any), 10. References, 11. Vitae, 12. Figure captions and 13. Tables.

Technical and reviews articles should have a similar structure, abstracts should not exceed 150 words. All articles should be in English, except news, reviews and reports what can be in Slovenian, Croatian or Serbian. Chapters should be arranged according to the standards ISO 2145 and ISO 690. Abbreviations must be explained at the first mention in the text.

First page of the article should contain the title of the article (max. 10 words), author(s)' name(s), institution and address. The corresponding author should be identified with an asterisk and footnote (the text in the footnote: to whom all correspondence should be addressed and added tel. and fax numbers, and e-mail). Second page should contain the abstract and keywords. An abstract of approximately 100 words should have the following structure: aims, scope and conclusions of the article.

Symbols and Units: Authors should follow the ISO 31 and IUPAC recommendations. Please note that all symbols should be written in italic; superscript and subscript are written normal.

Tables, figures and illustrations: Tables, figures and illustrations should be numbered consecutively and given a suitable caption, and each table, figure, and illustration should be given on a separate page and file. No vertical rules for tables should be used. Images should be of sufficient quality for the printed version (300 dpi minimum). Figures should be at good enough resolution (in EPS, JPEG, or TIFF format) to be assessed by the referees.

References: References should be numbered, and ordered sequentially as they appear in the text, methods, tables, figure captions. When numbered in the text, reference numbers should be in brackets, following the punctuation marks. If the cited reference has more then 3 authors, add the mark et al after the third author. References should be given in the following form:

[1] Bhatt Siddharta M. Energy audit case studies II - Air conditioning (cooling) systems. Appl Th Eng. 2000; 20: 297-307.

[2] American college of physicians. Clinical Ecology. An Int Med 1989; 111:168-78.

[3] Vivian VL, ed. Child abuse and neglect: a medical community response. Proceedings of the first AMA national conference on child abuse and neglect. 1984 Mar 30-31; Chicago. Chicago: American Medical Assotiation, 1985.

[4] Mansfieid LW. How the nurse learns which imbalance is present. V: Moidel HC, Sorensen GE, Giblin EC, Kaufman MA, eds. Nursing care of the patient with medical-surgical disorders. New York: Mc Grow-Hill, 1971: 153-60.

[5] Evaluation of the European Agency for Safety and Health at Work: http://osha.europa.eu/publications/other/20010315/index_1.htm (20. 12. 2006).

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