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Research of waste dump water mutagenicity of bacterial detection system SOS chromotest

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ABSTRACT

The paper deals with a possible use of the bacterial detection system of SOS chromotest to test mutagenicity of waste dump water checking the mutagenicity degree on real samples from Praksice waste dump, which is a controlled waste dump with mixed industrial, municipal and inert wastes. The waste dump surface water samples were taken from a no-name influent stream springing below the waste dump body between 2005 and 2009. After metabolic activation by microsomal fraction *in vitro*, medium to high mutagenicity was registered in all the samples. The SOS chromotest is assessed as an effective and economically acceptable method to check and determine the mutagenicity degree of contaminated water.

Key words | detection, detection systems, mutagenesis, mutagenicity, station, wastewater dump

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INTRODUCTION

Increasing demands of civilization that also manifest in the form of growing volumes of waste lead to the rising occurrence of harmful components in the environment. Their genotoxic effects exhibit in the form of more frequent occurrences of tumorous conditions, lowered reproductive capacities (reproductive loss, evolutionary defects) and worse health conditions of exposed organisms. For the past decades there has been a significant increase in the number of tumorous conditions, which in the Czech Republic represent the second most frequent cause of death; the issue concerns each of us (Fenclová 2007).

With regard to the high degree of pollution of the environment and working environment, methods of preventive evaluation of chemical contaminant genetic effects which permit identification and monitoring of their risk to people's health are important. The need for a complex approach to the issue of mutagenic activity of chemical substances has called for the development of not only special detection systems but also processing of general methodological procedures (Claxton *et al.* 1998; Rössner 2003; Punčochářová 2004).

Already in the mid-1980s a multi-stage checking of mutagenicity was designed to study a chemical substance's mutagenic activity. It was grounded in the use of various indicator objects, from microorganisms to mammals. It was the need to operatively consider the genotoxic effects of chemical substances that has led to the development of short-term detection systems which currently number several hundred. However, in many of them a low degree of reliability has been identified; in addition, the absolute difference of metabolic paths of prokaryotic and eukaryotic cells has led to the efforts to join the test advantages on microorganisms with possible monitoring of influence on mammalian cells (Ames *et al.* 1975; Hanawalt *et al.* 2000; Malachová *et al.* 2001; Šrám *et al.* 2004; Flegrová *et al.* 2008; Vojtková 2009).

Characteristics of locality of waste dump

The waste dump is located in the Czech Republic on the land on the boundary of the cadastral districts of Uherský Brod and Praksice, outside the built-up area (Figure 1). It is a dump with mixed industrial, municipal and inert waste. The dump is supplied with home waste from the catchment area, land waste, spoil and waste from building demolition.

According to the technical control it is a waste dump of S group – other waste of subgroup S-OO3 and S-OO1. The overall capacity of the dump is 418,355 m^3; with the annual banking of 35,000–40,000 t of waste. The dump is divided into four stages with calculated total lifespan till 2011. A part of the dump is a separate sector of subgroup S-OO1 with planned capacity of 9,000 m^3 to dump waste on the base of gypsum, stabilized waste and waste with sulfur

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Figure 1 | The waste dump Praksice – photomap (according to Geodis Brno, Czech Republic).

contents and sector S–OO3 with designed capacity of 15,000 m^3 to dump waste from asbestos. The waste dump designed capacity is 1,000 t of waste to one biodegradation process and annually two to three processes are planned.

In the past years, waste was dumped in different sites of the dump according to immediate possibilities and thus by a common mining-geological survey it is not possible to prove the quantity and exact type of waste in the specific site. The deposits of bioactive waste gradually decrease their volume (theoretically to 1/3), which causes irregular settlement of the dump surface. The waste dump does not have a sealing element from the bedrock, but the inner waste dump water is trapped by a drainage system and conducted into the collecting drainless ferroconcrete pit. The natural bedrock does not have sealing capacities which would substitute artificial sealing elements and thus the waste dump closure is made from geotextile layers of stock weight 800 g/m^2, next by PEHD foil 1.5 mm thick as a sealing element for reclamation layers and a geotextile layer of stock weight 500 g/m^2.

METHODS

Description of SOS chromotest method

The SOS chromotest is a bacterial detection system permitting evaluation of mutagenic activity of substances based on the capacity of the tested substances to induce the SOS repair system. Start-up of repair systems in a cell influences its further development – after successfully finished repairs, the cell may further develop in a normal way; in incomplete repairs serious changes in the cell genome occur that can lead all the way to genetically hereditary mutations or carcinogenic transformations. If the genetic structure damage is too large and reparations are not possible, the cell dies. The induction activity degree is assessed by means of a biochemical analysis of β-galactosidase induction, which is compared with a constitutively forming alkaline phosphatase. β-galactosidase induction occurs if a mutagen causes DNA damage in the form of a lesion, which activates RecA protein. The active form of RecA protein breaks the LexA repressor and thus releases SOS genes, to which sfiA gene also belongs, in whose operon the gene lacZ is also combined. Release of sfiA operon permits transcription of lacZ gene and a follow-up formation of β-galactosidase (Figure 2) (Vojtková 1989).

For this test a special strain of *Escherichia coli K 12 PQ 37* is used, which was developed in 1981 by Dr M. Hofnung (Pasteur Institut Paris). This strain was derived from the original strain of *Escherichia coli GC 4436* and it has the following genotype: F^-, thr, leu, his4, pyrD, thi, galE, galK, lacU169, srl300 :: Tn10 (Tc^r), rpoB (Rf^r), rpsL (Sm^r), sfi-A :: Mud (Ap, Lac)c-ts, rfa, uvrA, trp :: Muc^+, Pho^c. In the strain of *Escherichia coli PQ 37*, the genotype is changed especially by the presence of operon fusion sfiA :: lacZ for the formation of β-galactosidase, namely by insertion of a defective Mud (Ap,lac)c-ts prophage into sfiA operon of the host cell. The prophage gene lacZ is distinguished by the loss of its own promoter and its transcription is thus completely dependent on the promoter of bacterial sfiA gene. As a result of deletion of lacU 169 in the strain of *E. coli PQ 37* the own lactose operon is defective, and thus the prophage gene lacZ is solely responsible for the formation of β-galactosidase, whose transcription is conditioned by the SOS system induction (Quillardet *et al.* 1982).

Principle of SOS-chromotest

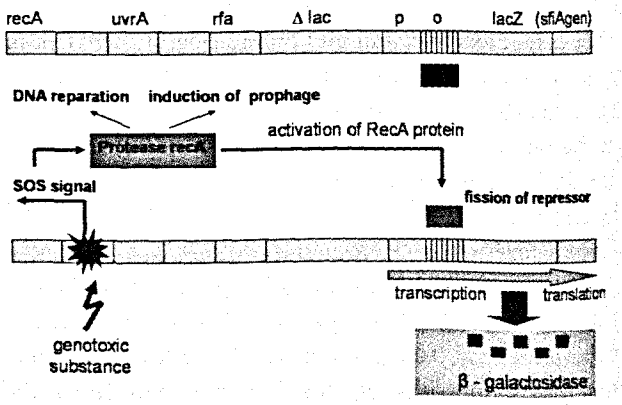

Figure 2 | Diagram of a SOS chromotest.

Better sensitivity of the detection strain to various groups of mutagens was also achieved by certain accessory mutations. By deletion of uvr gene and mutation of uvrA, the strain excision repair system was disabled. The mutation rfa leads to the disruption of a lipopolysaccharide membrane, which increases its permeability for certain other groups of mutagenic substances, such as aflatoxins, PAH (Quillardet *et al.* 1982; Vojtková 2000).

Based on the SOS chromotest principle, the SOS Chromotest Kit is produced and commercially supplied, containing all necessary agents and material permitting detection of genotoxic samples also in non-specialized laboratories in several hours. By means of the kit, the SOS chromotest is carried out on microtitration plates, into whose wells samples of the tested substance are gradually introduced in the required concentrations, checks, bacterial suspension or S9 fraction in the execution of the test with metabolic activation. The final level of genotoxicity can be evaluated visually according to the colour intensity, which corresponds to the degree of mutagenicity of the observed sample (Sezimová 2006; Vojtková 2009).

To test progenotoxic substances whose effect manifests after biotransformation in the organism, enzymatic liver microsomal extract – S9 fraction is used to model the metabolic paths (Vojtková 1989, 2009; Malachová *et al.* 2001; Stiborová 2005).

The microsomal fraction S9 is made up by an enzyme complex bound onto NADPH by cytochrome-reductase and is obtained from vertebrates liver (rodents) or fish pretreated by microsomal enzyme inductors. Such liver is then richer in certain enzymes, in monooxygenase in particular, which activates premutagens into metabolites with a genotoxic effect. The postmitochondrial fraction S9 may be obtained by centrifugation of liver tissue homogenate at 8,700 revolutions for 20–30 min. Subsequently, supernatant gets rid of the cell debris (i.e. mitochondrial nuclei, lysosomes, undamaged cells), and then contains only microsomes (Vojtková 2000, 2009; Punčochářová 2004; Stiborová 2005).

Sample collection

Waste dump waters sampled between 2005 and 2009 were tested by the SOS chromotest with metabolic activation by means of liver microsomal fraction S9. For testing, raw samples were used, taken from the surface flowing water springing under the waste dump body – raw (labelled as n) and diluted 0.5n (50%), 0.25n (25%), 0.125n (12.5%) and 0.0625n (6.25%).

RESULTS

In the course of waste dump water samples, a genotoxic effect in the form of positive results of detection of mutagenic action was identified in all the samples – both raw and diluted in the above stated concentrations. To be well-arranged, the obtained results are classified according to the year of sampling in the following charts; for better clarity, the sample induction activity in the individual years is separately compared and statistically assessed.

It is apparent from the obtained SOS chromotest results, assessing mutagenic activity of samples taken between 2005 and 2009, that there is a gradual increase in the values of the demonstrated induction activity. In 2009, the mean value of induction factor of an undiluted sample rose 16.49 times, which when compared with 2005 is a 1.35 times higher value, with 2006 it is a 1.32 times higher value, with 2007 it is a 1.25 times and with 2008 it is a 1.02 times higher value (Figures 3 and 4). This trend can be explained by a yearly increase in the quantity of dumped waste of about 40,000 t as well as by a rise in the mixed waste quantity where it is not always possible to distinguish the individual components of the waste, including their toxic effect. Comparing the test results in the individual periods, the highest values of mutagenicity were registered in the samples of 08-2008, where a multiple increase in the induction factor was identified as opposed to check values in which case the values of mean induction value rose two-times in an undiluted sample and reached the top values in the overall tested period.

The results proved the increasing mutagenic effects of water springing under the waste dump body due to the waste disposal process when a significant genotoxic effect

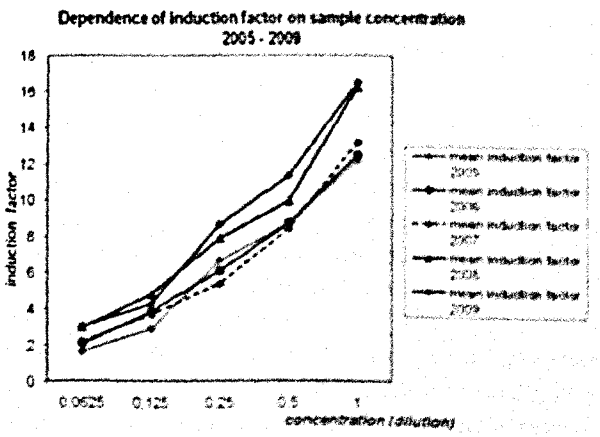

Figure 3 | Comparison of mean dependence of induction factor on sample concentration between 2005 and 2009.

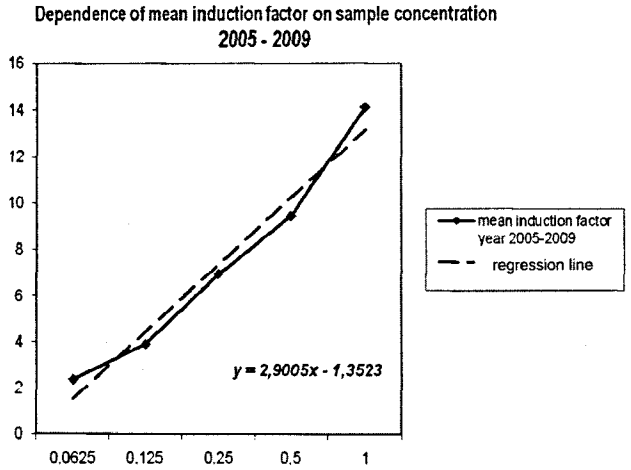

Figure 4 | Comparison of the average induction factor and concentration of the samples from 2005 to 2009 with regression line showing a linear relationship.

in the form of DNA cell degeneration begins to show already at dilution 6.25% (Figure 3). As a result of the degradation changes there is unambiguously disrupted stability of the cellular cycle; the final toxic effect may lead all the way to tumour transformation of tissue cells, which was studied by Kůsová *et al.* (2004).

CONCLUSION

This study demonstrates the possibility of using other tests to determine the degree of mutagenicity of water contaminated.

The SOS chromotest results, with metabolic activation on water samples springing under the waste dump body, unambiguously confirmed that the existing waste dump has strongly negative impact on the environment, in particular for water and soil organisms, including negative impacts on the grown commodities in the locality.

Utilization of the combination of microorganism detection system and activation mixture of S9 liver homogenate provides basic information on the influence of mammalian biotransformation processes on the tested substance mutagenicity. The introduction of testing with metabolic activation issued from the knowledge on genotoxic effects of many xenobiotics after metabolic conversion in the mammalian organism as the chemical substance biotransformation processes in the mammalian organism takes place under the action of enzymatic system of liver and organs securing the tissue exchange; in some cases primarily mutagenic substances are detoxicated in the mammalian organism. The complexity of the metabolic reaction participating in the activation of chemical substances only confirms new knowledge on the differences in metabolic paths, characteristic of the individual parts of the mammalian organism. Selecting the individual tests it is then necessary to take into consideration the detection capacities of the individual tests in a way that tests with similar information values were not used within the system.

The stated method of metabolic activation significantly widened the detection possibilities of the SOS chromotest, which can be viewed as a progressive acceptable method to determine the degree of mutagenicity of contaminated water.

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