

# TOXICITY RISK ASSESSMENT OF SULFUR AND PAHs IN SOIL SURROUNDING A COAL-FIRED POWER PLANT

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## Introduction

Coal is still one of the most important energy sources in many parts of the world, primarily those lacking pollution-control technology, thus profoundly affecting global atmosphere chemistry. Being the most complex geological material, its composition encompasses organic matter, water, oil, methane gas, and virtually all the elements in the periodic table, together with a wide variety of minerals (Finkelman, 1985; Radjenovic, 2006). Coal combustion residues contribute to the trace elements inventory in the atmosphere (Clarke and Sloss, 1992; Querol, 1995). The 1990 U.S. Clean Air Act Amendments list the following hazardous trace elements (HTE), As, Be, Cd, Co, Cr, Hg, Mn, Ni, Pb, Sb, and Se, as key toxic air pollutants (U.S. EPA, 1990). Low-quality coals are characterized by high ash, high sulfur, and high trace element contents. The main problem with sulfur in coal is its contribution to acid rain. As the total sulfur in coal varies in the range of 0.2-11%, commonly 1-3%, and due to stringent environmental regulations, various physical, chemical, and microbiological methods have been developed to remove it from flue gases (Radjenovic, 2004). Furthermore, polycyclic aromatic hydrocarbons (PAHs), formed during the fuel combustion processes, are organic pollutants that can be found in flue gas as well as in fly ash particles (Ravindra et al., 2008). Considering the fact that some of PAHs are highly mutagenic and carcinogenic (Sun et al., 2006; Yoon et al., 2007), an understanding of these compounds is required in addition to a reduction in their emission.

In Croatia, domestically mined Istrian and Dalmatian coals had been used in the Plomin coal-fired power plant (Plomin CFPP) situated in the city of Plomin (North

Adriatic, Istria), from its start in 1970 till the late 1990s. Amongst them, the Raša coal was exceptional in world terms as it contained as high as 13.05% sulfur (Valković et al., 1984a; White et al., 1990; Sinninghe Damsté et al., 1999; Chou, 2012). The Raša coal was reported by Chou (1997), together with coals from south China, as a member of the superhigh-organic-sulfur (SHOS) coals which are highly enriched in organic sulfur, usually in the range 4-11%. Also, the Raša coal has been known for high U values, averaging as high as 207 mg/kg (Valković et al., 1984b).

The environmental influence of the Plomin CFPP has been studied from several viewpoints so far. Marović (1985) found that the uranium activity concentration measured in the Raša coal was 250-300 Bq/kg, what was 10-15 times more than the then average value. Lokobauer et al. (1997) investigated radon concentrations in local old and new houses, as the slag and ash waste was used for the construction purposes. Marović et al. (2004, 2006) showed that remedial actions at the Plomin CFPP were followed by an improvement of the radiological environmental situation. Regarding the Plomin bay sediments, Oreščanin et al. (2002, 2009) found neither cytotoxic nor mutagenic effects in them.

Hazard and risk assessment of polluted soils is usually performed by means of physical and chemical measurements; however, biological assessments are useful to integrate the effects of all bioavailable contaminants and their interactions. The use of a set of tests on species at different levels of biological organizations to complement physico-chemical analyzes has been recommended for the evaluation of risk assessment (Oreščanin et al., 2002; Ternjej et al., 2013). Therefore, the main objectives of this study were: (1) to investigate the spatial (site specific) distribution of sulfur, PAHs, and trace elements in topsoil around the Plomin CFPP taking into account direction of the prevailing winds in the area; and (2) to assess cytotoxic and genotoxic potential of water extracts of this soil.

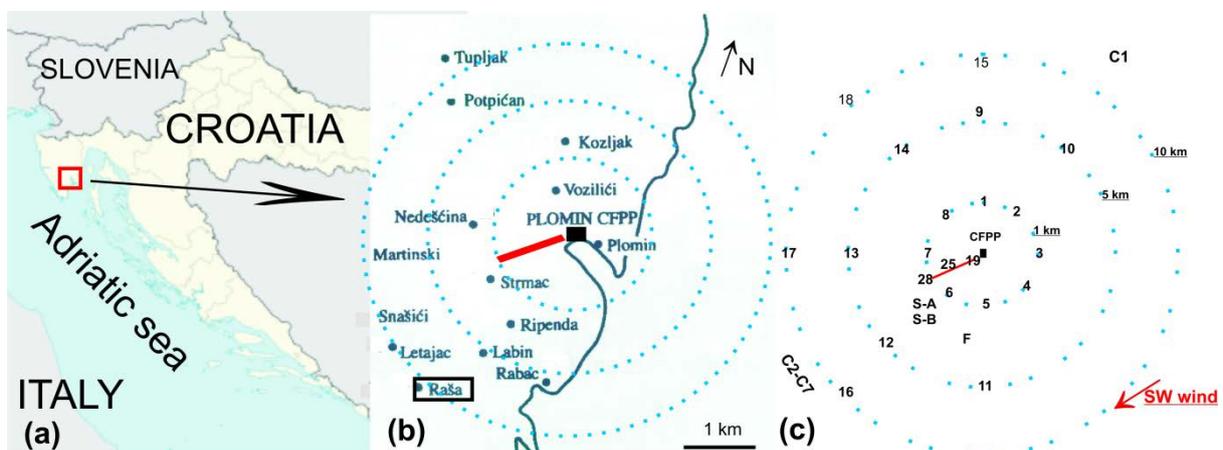
## **Methodology**

### **Site description and sampling strategy**

Medunic et al. (2014) reported preliminary results of a study carried out on sixteen topsoil samples taken within radii of 1, 5, and 10 km around the Plomin CFPP, together with ten topsoil samples taken along the prevailing wind direction (south-westward, SW), also annotated as the pollution gradient (PG) samples collected at distances between 100 m and 1 km from the Plomin CFPP, as well as on two control samples C1 and C2, outside the 10 km radius (Figure 1). Since the results showed marked sulfur soil pollution in the SW quadrant of the locality (i.e. in the prevailing wind direction), an additional five control topsoil samples (C3-C7) were taken outside the 10 km radius of the SW quadrant (Figure 1), together with several top- as well as subsoil samples positioned between the 1st and 5th km circles (i.e. the sites S and F presented on Figure 1). At each location, after removing the top few centimetres of vegetative cover, approximately 1 kg of topsoil sample was taken

with the sampling shovel. Two randomly selected parts of the recently dumped fly-ash and bottom ash waste; rather heterogeneous regarding colour and structure (near the CFPP) were also sampled (samples A1 and A2, predominantly of grey and dark tone, respectively). All soil samples were wet-sieved to  $-63 \mu\text{m}$ , then air-dried and disaggregated in an agate mortar for further analysis. Statistical analysis of the PIXE measurements reported here was carried out on three groups of samples defined as follows: (1) SW – 6, 7, 16, and the PG samples (19-28); (2) non-SW – 1, 2, 3, 4, and 8; and (3) C – control samples (C1-C7).

The study area belongs to the classical karst terrain composed of Dinaric carbonate rocks, overlain by brown soil classified as eutri-chromic cambisol (Miko et al., 2003). During the Early Paleocene, fresh-water and brackish 'Liburnian' strata, otherwise rich in radionuclides contained in the associated coal seams, were discordantly deposited in isolated basins (Barišić et al., 1994). Afterwards, they were subjected to a major transgression which resulted in marine deposition of limestones and flysch. A major feature of the area is local coal mines (Labin, Raša, Strmac, Ripenda, Tupljak, and Potpićan) which were finally closed some fifteen to twenty years ago. Coal mining in the area dates back to in the second half of the 18th century. Slag and ash dumps are scattered around the former mine sites (site S-A) as well as around the CFPP, and are covered with soil and vegetation. Sampling point F (soil profile  $>100 \text{ cm}$  thick) is situated next to the newly built road extending from the city of Labin towards the north-east direction (i.e. towards the city of Rijeka).



**Figure 1. Geographical location of the study area (a) with sampling sites: (b) – sampling circles at 1, 5, and 10 km; red line represents the pollution gradient (PG) equivalent to south-westward (SW) wind (sampling points 19-28); (c) – sampling localities (sample numbers 15 and 18 were excluded from analyses because of different soil type).**

## Analytical methods

Major (Al, Si, K, Fe, Ca, and Mg), minor (Na, Ti), and several trace elements (Mn, V, Cr, Ni, Cu, and Zn) were measured by PIXE (particle induced X-ray emission) technique, which was performed at the Rudjer Bošković Institute Tandem Accelerator Facility. Pelletized soil and ash samples, together with two reference samples were mounted inside the vacuum chamber for the ion beam analysis. They were irradiated with 2 MeV protons accelerated with 1 MV Tandetron accelerator. The PIXE spectra were collected with two detectors: an SSD detector placed at 150° for the detection of low energy X-rays, and a Si(Li) detector placed at 145° for the detection of higher energy X-rays. The protons backscattered from target (RBS) were collected by an Ion implanted silicon detector simultaneously with the PIXE spectra. The RBS spectra were analyzed with simulation software SIMNRA to determine concentrations of major elements which are invisible to the PIXE detectors. The estimation of major element concentrations was then used as an input matrix for the thick target quantification procedure in analyzes of the measured PIXE spectra by the GUPIXWIN software.

Taking into account some shortfalls in PIXE analyzes associated with spectral interferences (Medunic, 2014), samples were also analyzed by ICP-MS. Subsamples (0.1 g) of soils and ashes were subjected to total digestion in the microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) in two step procedure consisting of digestion with a mixture of 4 mL nitric acid (HNO<sub>3</sub>) - 1 mL hydrochloric acid (HCl) - 1 mL hydrofluoric acid (HF) followed by addition of 6 mL of boric acid (H<sub>3</sub>BO<sub>3</sub>). Indium (1 µg/L) was added to the digested samples as the internal standard. Multielement analysis of the prepared samples was performed by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) using Element 2 instrument (Thermo, Bremen, Germany). Typical instrument conditions and measurement parameters used throughout the work were reported previously (Fiket et al., 2007; Cukrov et al., 2008). Good agreement between the analyzed and certified concentrations within their analytical uncertainties was obtained for all elements for the both analytical methods (PIXE ± 30%, and ICP ± 10%).

Determination of polycyclic aromatic hydrocarbons (PAHs) followed a modified procedure by Giger and Schaffner (1978). Briefly, aliquots of 5 g of sediment samples were extracted with dichloromethane using accelerated solvent extraction. The total extract was purified using gel-permeation chromatography using Envirogel™ GPC (Waters, USA) clean-up column. The purified extracts were further cleaned up on a column of fully active silica gel. The aromatic fraction, containing all PAHs, was obtained by elution with dichloromethane. The eluate was evaporated to dryness, reconstituted in cyclohexane and analysed by high-resolution gas chromatography/mass spectrometry (HR GC/MS) as described earlier in Mikac et al. (2011). Briefly, the GC/MS analyses were performed using a GC/MS system consisting of an Agilent 7890A gas chromatograph, equipped with a 20 m × 0.2 mm × 0.25 µm HP-5 fused silica column, and a quadrupole mass selective detector

(Agilent, model MSD 5975C inert XL EI/CI). The column temperature was programmed from 50 °C to 300 °C at 5 °C min<sup>-1</sup> and the samples were acquired in full scan mode. The samples were analyzed for 16 nonsubstituted PAHs, which were quantified from the reconstructed mass chromatograms using their corresponding molecular ions. The quantification of the individual PAHs was performed using suite of deuterated PAH as internal standards, which were added to the extracts before the GPC clean-up. The accuracy of the method was tested using standard reference material RTC-CRM 105 (RT Corporation Ltd, UK). The detection limits for all individual PAHs were below 5 ng/g, while reproducibility varied from 10 to 20%.

Soil extracts were prepared by mixing 20 g of dry soil samples with 20 ml of double distilled water for 24 h at room temperature (22°C). Following that, additional 20 ml of distilled water was added and after 24 h of agitation, the suspension was centrifuged. Extract was sterilized by filtration through Millipore filter with pore size of 0.45 µm and kept frozen until testing (Oreščanin et al., 2002). CCO cells, an established fibroblast-like cell line (ATCC: CRL-2772), was used for the study of cytotoxicity and genotoxicity. Cells were cultivated in DMEM medium (Sigma, St. Louis, MO, USA) containing 10% inactivated fetal bovine serum (FBS) (GIBCO, Paisley, Scotland, UK) at an optimal growth temperature of 30 °C and 5% CO<sub>2</sub> atmosphere. The cytotoxicity of soil leachates was evaluated by WST-1 assay while genotoxicity was evaluated by alkaline Comet assay as previously reported by Ternjej et al. (2013). The obtained data were statistically analyzed by commercial software Statistica 12.0 (StatSoft, Tulsa, USA). Differences between controls and exposed concentrations were considered statistical significant at P<0.05.

Since the aim of this study was to evaluate whether the samples of soil collected at different distances from the Plomin CFPP show any genotoxic properties, to determine their primary DNA-damaging potential, the alkaline comet assay on channel catfish ovary (CCO) cell line was performed. The analysis was carried out according to the standard protocol (Singh et al., 1988), with minor modifications. Agarose microgels (duplicate slides per sample) were prepared using the standard three-layer procedure. All chemicals were of analytical grade and purchased from Sigma Chemical Co. Samples of the CCO cell cultures (2x10<sup>6</sup> cells/ml, V=10 µl per slide) were mixed with 100 µl of 0.5%-LMP (Low Melting Point) agarose and put on a pre-prepared layer of 0.6%-NMP (Normal Melting Point) agarose. This layer was covered with 100 µl of 0.5% LMP agarose and left to solidify on ice. Positive control was hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is able to produce an excess of DNA single- and induce DNA double-strand breaks (Olive and Banáth, 2006). The treatment of CCO cells embedded into an agarose microgel with 50 µM H<sub>2</sub>O<sub>2</sub> lasted for 10 minutes and was performed on ice (according to Ternjej et al., 2013). Following the preparation, microgels were subjected to a lysis (for 1 h at 4 °C) in accordance with standard protocols (2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris-HCl, 1% N-lauroylsarcosine sodium salt, pH 10 with 1% Triton X-100 and 10% dimethyl sulfoxide). Alkaline denaturation and electrophoresis (1 V/cm, 300 mA)

were carried out in a freshly prepared electrophoretic buffer (300 mM NaOH, 1 mM Na<sub>2</sub>EDTA, pH 13.0) for 20 min each, at 4 °C and under a dim light. After electrophoresis, the samples were washed three times with a neutralization buffer (0.4 M Tris-HCl, pH 7.5) at five-minute intervals and stained with 100 µl of ethidium bromide (20 µg/ml) for 10 minutes. The slides were then stored at 4°C in humidified sealed containers pending analysis. The scoring was performed using an image analysis system (Comet Assay IV, Perceptive Instruments Ltd., UK) attached to a fluorescence microscope (Olympus, Japan) equipped with appropriate filters, under a 200x-magnification. A total of 200 comets per sample were measured (100 comets per each replicate slide). Comets were randomly captured at a constant gel depth, thereby avoiding gel edges, occasional dead cells and superimposed comets. To evaluate DNA damaging potential of tested samples, the parameter “tail intensity” (DNA% in the comet tail) was selected, which is commonly considered the most appropriate indicator of DNA damage, as it points to the number of DNA breaks (Olive, 1999; Collins, 2004). Also, beyond a critical quantity of damage, tail intensity increases, rather than comet tail length. Statistical analyses were performed using Statistica Ver. 12 Software (StatSoft Inc., Tulsa, USA). The extent of DNA damage, as recorded by the alkaline comet assay, was analyzed for the main descriptive statistical parameters. Furthermore, the data were transformed in a logarithmic manner to normalize their distribution and equalize the variances. Further comparisons were performed using the one-way analysis of variance followed by *post-hoc* Schéffe test for the calculations concerning pair-wise comparisons. Statistical decisions were made at a significance level of P<0.05.

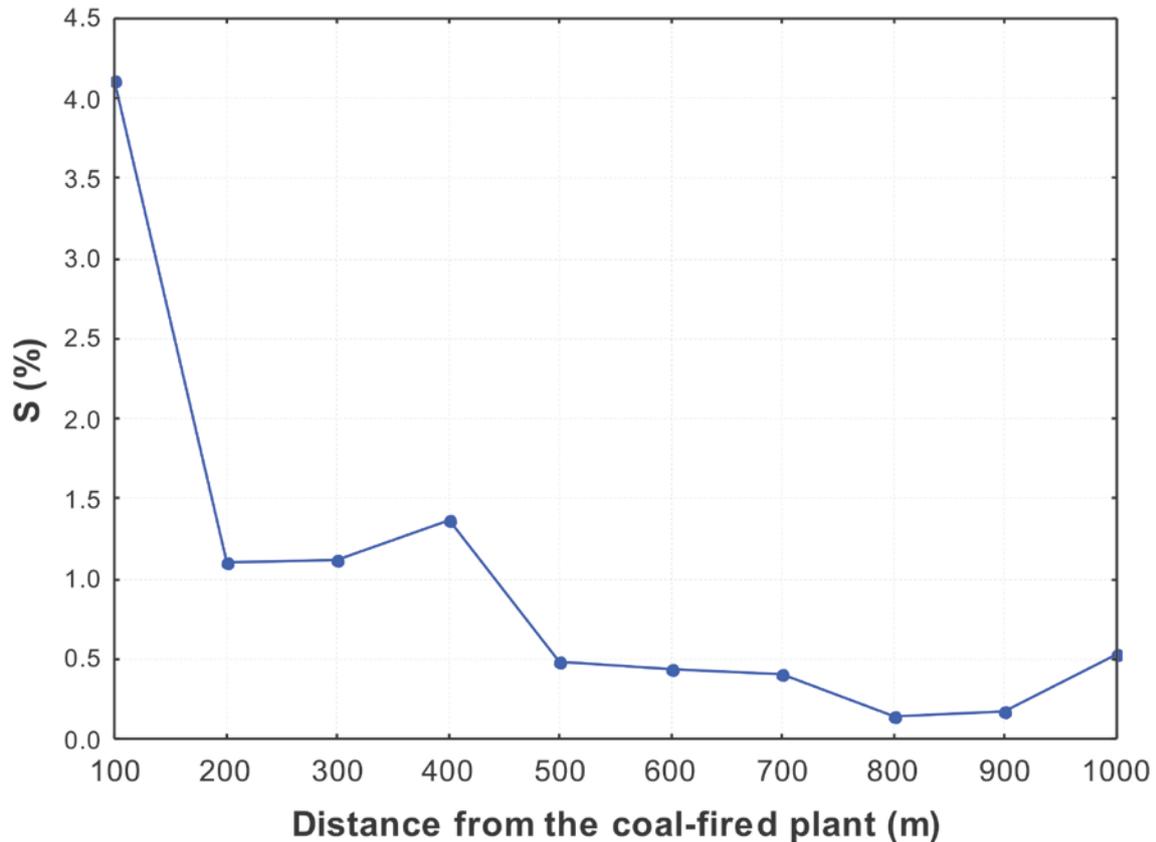
## Results

Elemental examination of soil around the Plomin CFPP shows it to be severely polluted with sulfur (Table 1).

**Table 1. Basic statistical parameters of sulfur (%) in three soil groups**

Mean	Std. Dev.	Min - Max
0.074 (C)	0.027	0.036 – 0.106
0.141 (non-SW)	0.042	0.121 – 0.216
0.928 (SW)	1.052	0.144 – 4.108

The mean sulfur content of soil samples taken from the SW quadrant is approximately 12.5 times greater compared to the control (‘C’) sites. This is mainly due to the sulfur content in the samples 19-22 situated nearest to the CFPP (100-400 m), along the PG line (Figure 2).



**Figure 2. Distribution of sulfur in the pollution gradient (PG) samples along the SW wind direction (scaling scheme identical for all samples).**

Comparison of major and minor element values (PIXE), according to the soil groups (C, SW, and non-SW), is depicted in Figure 3. Box plots show the so-called dilution-effect of a strange component (coal and coal combustion residue) in soil, as geogene elements (Al, Si, Na, K, Ti, and Fe) are more or less decreased in the SW group compared to the C group. On the contrary, Ca is characterized by generally higher values in the SW group compared to the C and non-SW groups. This could be ascribed to the possible CaO added to the fuel to protect the stack from damaging effects of the high S levels in the fuel (Clarke and Sloss, 1992).

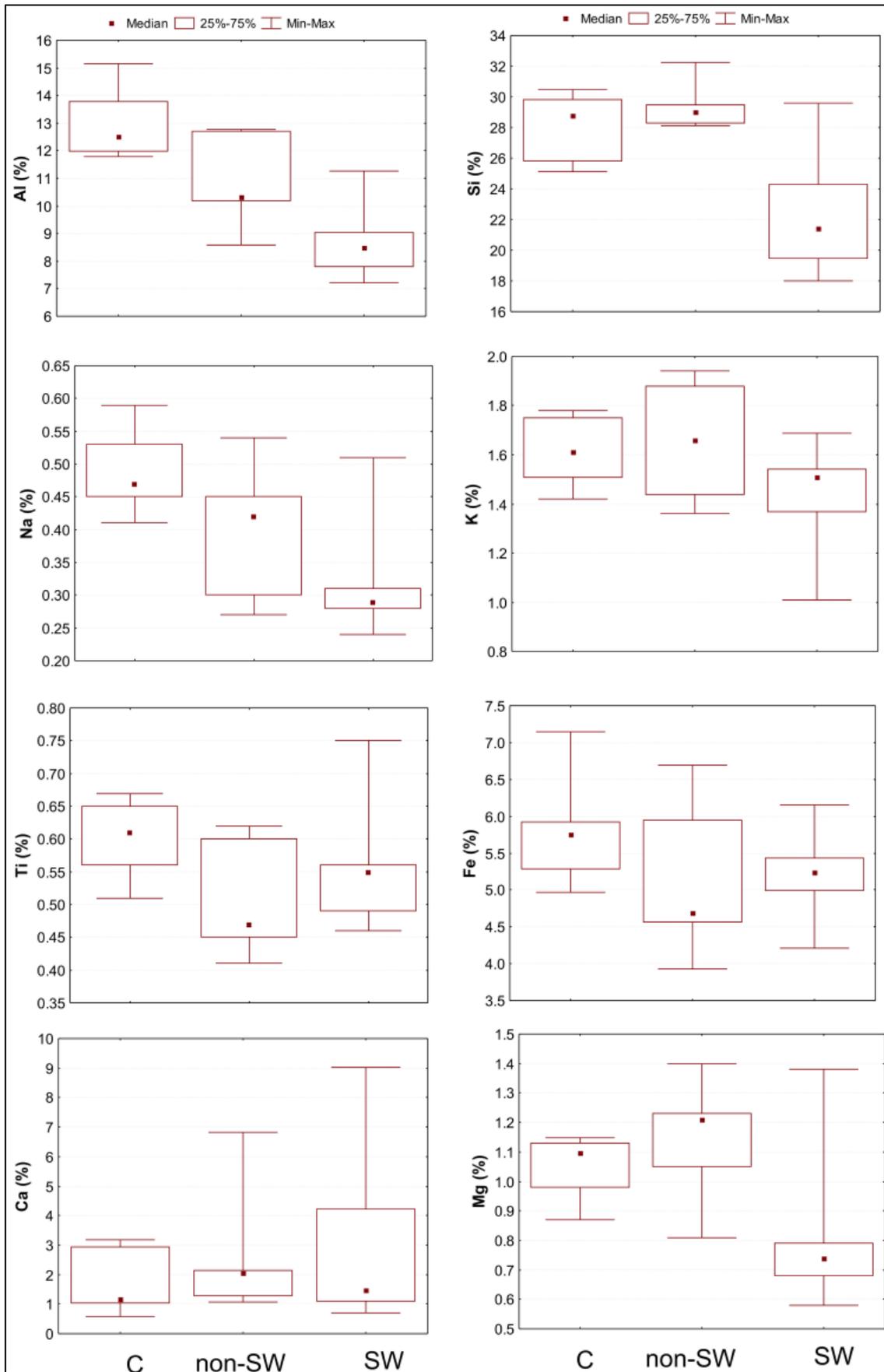


Figure 3. Comparison of major and minor element values (PIXE) in three soil groups.

Also, comparison of minor and trace element values (PIXE) in soil groups is depicted in Figure 4. Here, box plots show that V, Cu, and Zn, elements of mostly anthropogenic origin, have higher values in the SW as well as non-SW groups compared to the C group. This could be described by the CFPP's influence on surrounding soils as Limić and Valković (1986) examined a coal seam enriched in many trace elements, and they reported mean ranges of V, Cu, and Zn (in mg/kg) as follows: 341-1530, 26.8-50.9, and 11.2-16.9, respectively.

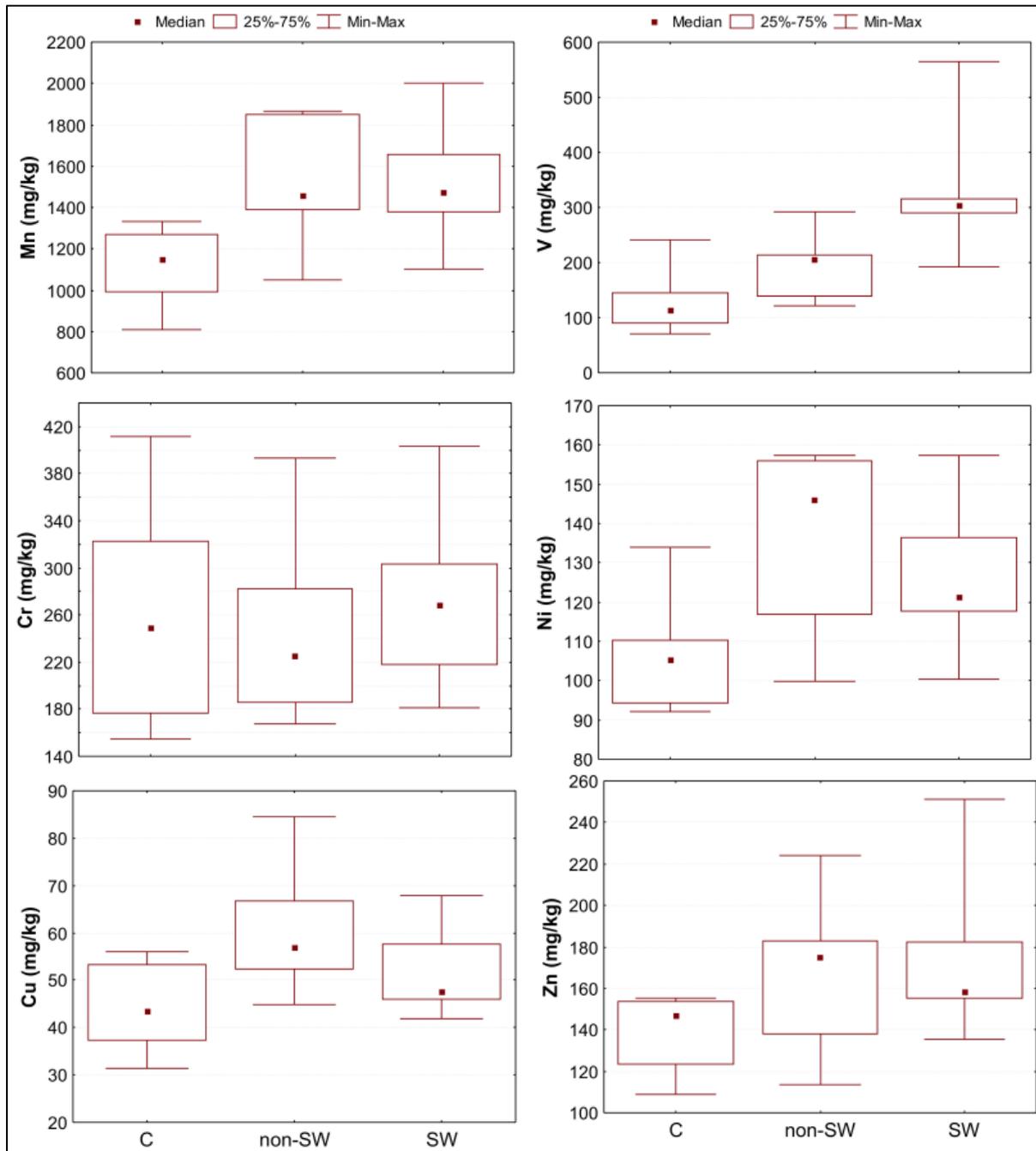


Figure 4. Comparison of minor and trace element values (PIXE) in three soil groups.

Since the analytical scientists from various branches have come to view PIXE as a viable analogue of the established ICP method, analytical results in this study were checked and found to be highly comparable. Hereby, arithmetic means of PIXE/ICP ratios of element values ranged from 0.9 to 1.4, while their standard deviations ranged from 0.1 to 0.2. Table 2 presents some results of the ICP measurements in selected samples.

**Table 2. Element values (ICP) in soil and ash samples (mg/kg)**

Element	Soil (C5)	Soil (F 0-10 cm)	Technogenic soil (S-A)	Ash (A2)
Mo	2.72	2.81	3.98	20.9
Cd	1.68	1.60	2.95	4.08
Sn	4.83	5.13	23.2	8.11
Cs	7.44	9.74	3.19	13.8
Pb	41.8	44.8	201	94.0
Sb	1.48	1.85	3.34	16.9
Ba	345	333	389	4072
Cu	31.3	35.8	1692	139
Zn	104	105	6052	356
As	21.9	22.9	9.2	75.2
Se	0.81	0.94	3.62	15.8

During this study, special attention was paid to PAHs as they are typical products of combustion processes. The total concentrations of PAHs in topsoils around the Plomin CFPP varied from 31 ng/g in the control (C) samples collected outside the 10-km zone, to 13,535 ng/g at a distance of 100 m from the CFPP (Table 3).

**Table 3. Basic statistical parameters of PAHs in soil (ng/g)**

Mean	Std. Dev.	Min - Max
122 (Control)	128	31 – 213
1,291 (1 km circle)	1,745	131 – 4,487
6,917 (Pollution gradient)	4,175	2,643 – 13,535

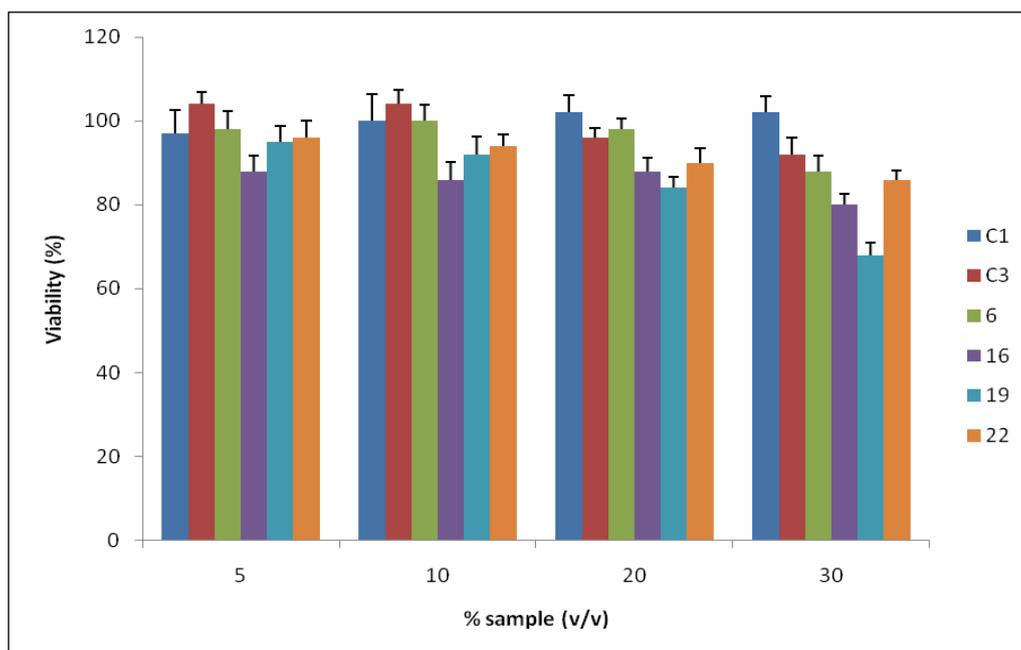
As can be seen from Table 3, the concentrations of PAHs are highly dependent on the two main factors determining dissipation of emissions from the CFPP – the distance from the facility, and the wind direction. Statistical analysis showed statistically significant differences between the PAH levels within 1 km zone from the CFPP and the control sites located outside the 10-km zone. However, the highest average concentrations were observed along the 1 km-long transect (PG samples), extending from the CFPP in the SW direction (Figure 5).



**Figure 5. Distribution of PAHs (ng/g) in the pollution gradient (PG) samples along the SW wind direction.**

This can be explained by the predominant SW wind direction in the area. Nevertheless, PAH concentrations decreased rapidly with distance (D), so that their values dropped to about 200 ng/g at a distance of 1 km.

The effects of different soil leachate samples collected around the Plomin CFPP on CCO cells, determined by WST-1 assay, are presented in Figure 6. The most pronounced and dose-dependent cytotoxicity ( $P < 0.05$ ) to CCO cells was found for sample 19, which correlated with the highest sulfur and PAHs concentrations.



**Figure 6. Viability of the CCO cells.**

Results of the evaluation of DNA damage inflicted in CCO cells after *in vitro* exposure to each tested sample are shown in Figure 7. Also presented are detailed intergroup comparisons and statistical evaluations by ANOVA with *post-hoc* Schéffe test.

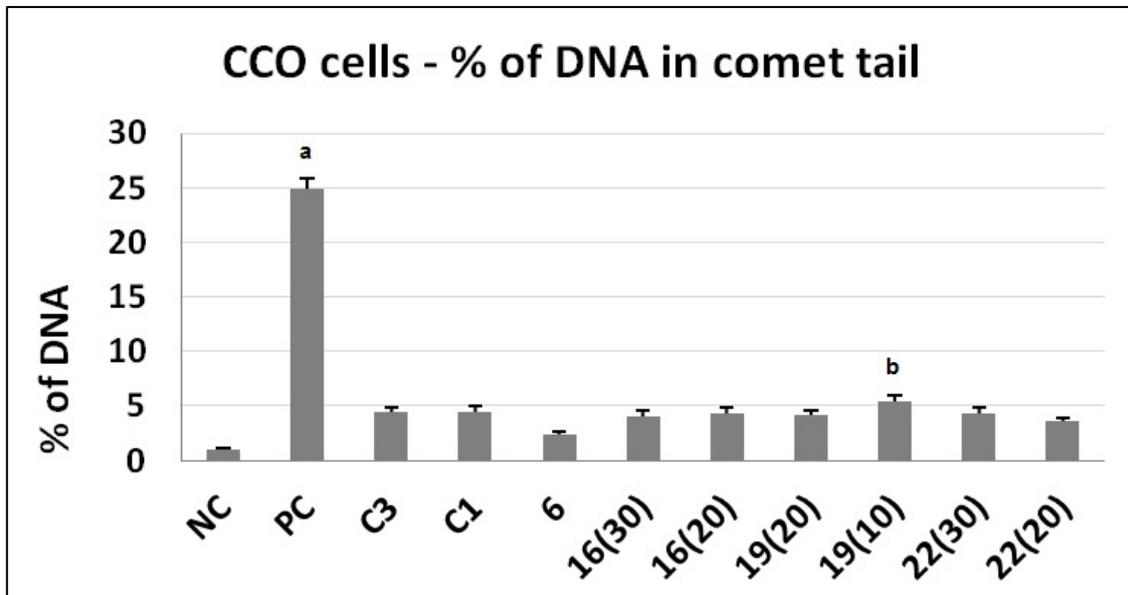


Figure 7. Primary DNA damage as estimated by the alkaline comet assay in treated CCO-cells. Results are shown as mean value and standard error of the mean. NC – negative control; PC – positive control; parameters of the alkaline comet assay were analyzed by measuring 200 comets per each sample. Significance of the results obtained between groups was studied using ANOVA with *post-hoc* Scheffé test. Significant difference ( $P < 0.05$ ): a vs. negative control; b vs. sample 6.

## Discussion & Conclusions

The Plomin CFPP is composed of two boiler units, one built in 1969, and another one built in 2000. They generate a total of 330 [megawatts](#). Slag and ash have been deposited since 1971, covering the area of approximately 120,000 m<sup>2</sup> (Marović et al., 2006). Since the most interesting findings in this study are elevated values of sulfur (Figure 2) and PAHs (Figure 5) in the PG samples (point 19 (Figure 1) in particular), they can be described by their position near the fence of the CFPP's property. Sample point no. 19, which is the most polluted with sulfur (4.108%) and PAHs (13,535 ng/g), belongs to the part of the terrain which had been subjected to several remedial actions more than 10 years ago (Marović et al., 2004, 2006). It could be assumed that the PG locality probably suffered from these actions, having probably received a certain amount of coal combustion waste. Also, the sulfur and PAHs distribution patterns could be related to the meteorological conditions affecting the fate of the emitted gases and airborne particulate matter. Concerning the highly

increased Cu and Zn values in sample S-A (Table 2), they could be attributed to underlying waste of an ex-foundry Strmac, which was closed some 60 years ago.

As to the composition of PAHs in analyzed samples, the low molecular weight (LMW) PAHs clearly dominate in highly polluted samples, whereas the high molecular weight PAHs prevailed at the control sites. It should be stressed that there have been two distinct sources of PAHs in the Plomin CFPP. The highly prominent source are high temperature combustion processes, resulting in slag and fly ash, while the additional contribution could have derived from unburnt coal from a large open coal storage situated near the CFPP. According to recent study by Dong et al. (2012), the enhanced contribution of LMW PAHs in the most polluted soils (Figure 5, sample 19) should be interpreted as the prevailing contribution of pyrolytic sources. It is interesting to note that the analyses of the ash samples showed approximately the same concentration levels of PAHs (10,829 ng/g) like the most polluted soil samples in the recent few years. This result can be interpreted to be the consequence of the replacement of the Raša coal with imported coals having much better quality and much lower emission characteristics regarding sulfur and PAHs.

This study demonstrated that the CCO cell line proved to be suitable for the purpose of the investigation of the potential cytotoxic and genotoxic effects of contaminated soils from the Plomin CFPP. The obtained cytotoxic effects of water soil leachates on the CCO cells indicated that these effects could be related to the increased level of sulfur as well as PAHs.

The values of comet parameters measured in the negative control indicate the low level of spontaneous damage to the DNA of CCO cells. As expected, the positive control (H<sub>2</sub>O<sub>2</sub>) induced a significant response in comet assay. The obtained results suggested that all tested soil samples induced a slight, but not statistically significant increase of primary DNA damage as compared to negative control. The highest primary DNA damage was observed (Figure 7) in the sample no. 19 (Figures 1, 2, and 5), which is the most polluted site as regards S and PAHs, whereas the lowest one was exhibited by the sample no. 6 (Figure 1), situated at the 1st km circle. Inter-group comparisons did not show statistically significant differences between damage levels measured in the majority of samples. Thus, the obtained evaluations indicate that the levels of DNA damage in treated CCO cells were within the acceptable boundaries, and that all tested samples collected near the CFPP did not induce genotoxic potency towards the cell type used for the purposes of this study. It is likely that the increase of DNA damage observed in treated cells could be attributed to pro-oxidative activity of the compounds contained in the tested samples and their ability to create reactive oxygen species. However, due to the complex composition of the samples, it is not possible to stress which particular compound was responsible for the effects observed. Polluted environmental samples are often of complex composition, with constituents that possess many different mechanisms of toxicity, and all of them could contribute to the damaging effect measured by the alkaline comet assay in single cells. To conclude, this study gives only a preliminary

insight into the genotoxicity profiles of the tested samples *in vitro*, and calls for further research, especially considering the fact that only one cell type was used for the analysis.

## References

BARIŠIĆ, D., PROHIĆ, E. & LULIĆ, S. 1994. Some problems of the radiometric mapping of carbonate terrains – an example from Istria (Croatia). *Geologia Croatica*, **47/1**, 73-82.

CHOU, C.-L. 1997. Geologic factors affecting the abundance, distribution, and speciation of sulfur in coals. In: YANG, Q. (Ed), *Geology of fossil fuels*, Proceedings of the 30th International Geological Congress: Part B, VSP, Utrecht, The Netherlands, **vol. 18**, 47-57.

CHOU, C.-L. 2012. Sulfur in coals: a review of geochemistry and origins. *International Journal of Coal Geology*, **100**, 1-13.

CLARKE, L. B. & SLOSS, L. L. 1992. Trace elements – emissions from coal combustion and gasification. IEACR/49. IEA Coal Research, London, 111 p.

COLLINS, A. R. 2004. The Comet assay for DNA damage and repair, principles, applications, and limitations. *Molecular Biotechnology*, **26**, 249–261.

CUKROV, N., CMUK, P., MLAKAR, M. & OMANOVIĆ, D. 2008. Spatial distribution of trace metals in the Krka River, Croatia. An example of the self-purification. *Chemosphere*, **72**, 1559-1566.

DONG, C.-D., CHEN, C.-F. & CHEN, C.-W. 2012. Determination of polycyclic aromatic hydrocarbons in industrial harbor sediments by GC-MS. *International Journal of Environmental Research and Public Health*, **9 (6)**, 2175-2188.

FIKET, Ž., ROJE, V., MIKAC, N. & KNIEWALD, G. 2007. Determination of arsenic and other trace elements in bottled waters by high resolution inductively coupled plasma mass spectrometry. *Croatica Chemica Acta*, **80**, 91-100.

FINKELMAN, R. B. 1985. Characterization of the Inorganic Constituents in Coal. *MRS Proceedings*, **65**, 71.

GIGER, W. & SCHAFFNER, C. 1978. Determination of polycyclic aromatic hydrocarbons in the environment by glass capillary gas chromatography. *Analytical Chemistry*, **50 (2)**, 243-249.

LIMIĆ, N. & VALKOVIĆ, V. 1986. The occurrence of trace elements in coal. *Fuel*, **65**, 1099-1102.

- LOKOBAUER, N., FRANIĆ, Z., SENČAR, J., BAUMAN, A. & SOKOLOVIĆ, E. 1997. Radon concentrations in houses around the Plomin coal-fired power plant. *Journal of Environmental Radioactivity*, **34 (1)**, 37-44.
- MAROVIĆ, G. 1985. Enhanced natural radioactivity around a coal-fired power plant. M.Sc. Thesis, Technological Faculty, University of Zagreb (in Croatian).
- MAROVIĆ, G., SENČAR, J., KOVAČ, J. & PRLIĆ, I. 2004. Improvement of the radiological environmental situation due to remedial actions at a coal-fired power plant. *Journal of Radioanalytical and Nuclear Chemistry*, **261 (2)**, 451-455.
- MAROVIĆ, G., SENČAR, J., BRONZOVIĆ, M., FRANIĆ, Z. & KOVAČ, J. 2006. Otpad vezan uz proizvodnju električne energije i proizvodnju mineralnih gnojiva (Radioactive waste due to electric power and mineral fertiliser production; In Croatian). *Arhiv za Higijenu Rada i Toksikologiju*, **57**, 333-338.
- MEDUNIĆ, G. 2014. The joy of using nuclear analytical tools in environmental studies. *Journal of Environmental Analytical Chemistry*, **1**:e103. doi: 10.4172/JREAC.1000e103.
- MEDUNIĆ, G., BOŽIČEVIĆ MIHALIĆ, I., KOPJAR, N., GAURINA SRČEK, V., CUCULIĆ, V. & OREŠČANIN, V. 2014. Sulphur soil pollution caused by a coal-fired power plant (Plomin, Croatia). In: Belviso, C., Saverio, F. & Gianossi, M. L. (eds) *Book of abstracts of International Conference on atmospheric dust (DUST 2014)*, 276-276.
- MIKAC, I., FIKET, Ž., TERZIĆ, S., BAREŠIĆ, J., MIKAC, N. & AHEL, M. 2011. Chemical indicators of anthropogenic impacts in sediments of the pristine karst lakes. *Chemosphere*, **84**, 1140-1149.
- MIKO, S., DURN, G., ADAMCOVÁ, R. et al. 2003. Heavy metal distribution in karst soils from Croatia and Slovakia. *Environmental Geology*, **45**, 262-272.
- OLIVE, P. L. 1999. DNA damage and repair in individual cells: applications of the Comet assay in radiobiology. *International Journal of Radiation Biology*, **75**, 395–405.
- OLIVE, P. L. & BANÁTH, J. P. 2006. The comet assay: a method to measure DNA damage in individual cells. *Nature Protocols*, **1**, 23-29.
- OREŠČANIN, V., FRANEKIĆ-ČOLIĆ, J., DURGO, K. & VALKOVIĆ, V. 2002. Investigation of mutagenic effect of metals in the Plomin bay sediments by modified preincubation ames assay. *Journal of Trace and Microprobe Techniques*, **20 (1)**, 69-77.
- OREŠČANIN, V., NAĐ, K., BARTOLINČIĆ, A. & VALKOVIĆ, V. 2009. Chemical profile of Plomine bay sediments. *Arhiv za Higijenu Rada i Toksikologiju*, **60**, 281-287.

- QUEROL, X., FERNÁNDEZ-TURIEL, J. L. & LÓPEZ-SOLER, A. 1995. Trace elements in coal and their behaviour during combustion in a large power station. *Fuel*, **74 (3)**, 331-343.
- RADJENOVIC, A. 2004. Sumpor u ugljenu (Sulfur in coal, in Croatian), *Kemija u Industriji*, **53 (12)**, 557-565.
- RADJENOVIC, A. 2006. Inorganic constituents in coal. *Kemija u Industriji*, **55 (2)**, 65-71.
- RAVINDRA, K., SOKHIA, R. & VAN GRIEKENB, R. 2008. Review: Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. *Atmospheric Environment*, **42**, 2895-2921.
- SINGH, N. P., McCOY, M. T., TICE, R. R. & SCHNEIDER, L. L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, **175**, 184–191.
- SINNINGHE DAMSTÉ, J. S., WHITE, C. M., GREEN, J. B. & de LEEUW, J. W. 1999. Organosulfur compounds in sulfur-rich Raša coal. *Energy & Fuels*, **13**, 728-738.
- SUN, P., WEAVERS, L. K., TAERAKUL, P. & WALKER, H. W. 2006. Characterization of polycyclic aromatic hydrocarbons (PAHs) on lime spray dryer (LSD) ash using different extraction methods. *Chemosphere*, **62**, 265-274.
- TERNJEJ, I., GAURINA SRČEK, V., MIHALJEVIĆ, Z. & KOPJAR, N. 2013. Cytotoxic and genotoxic effects of water and sediment samples from gypsum mining area in channel catfish ovary (CCO) cells. *Ecotoxicology and Environmental Safety*, **98**, 119-127.
- U.S. Environmental Protection Agency (U.S. EPA). 1990. Clean Air Act Amendments of 1990; 1st Congress (1989-1990); U.S. EPA: Washington, DC.
- VALKOVIĆ, V., ORLIĆ, I., MAKJANIĆ, J., RENDIĆ, D., MIKLAVŽIČ, U. & BUDNAR, M. 1984a. Comparison of different modes of excitation in x-ray emission spectroscopy in the detection of trace elements in coal and coal ash. *Nuclear Instruments and Methods in Physics Research* **B4**, 127-131.
- VALKOVIĆ, V., MAKJANIĆ, J., JAKŠIĆ, M., POPOVIĆ, S., BOS, A. J. J., VIS, R. D., WIEDERSPAHN, K. & VERHEUL, H. 1984b. Analysis of fly ash by X-ray emission spectroscopy and proton microbeam analysis. *Fuel*, **63**, 1357-1362.
- WHITE, C. M., DOUGLAS, L. J., ANDERSON, R. R., SCHMIDT, C. E. & GRAY, R. J. 1990. Organosulfur constituents in Rasa coal. In: Orr, W. L. & White, C. M. (eds.) *Geochemistry of Sulfur in Fossil Fuels*. Chapter 16, pp. 261-286. *American Chemical Society Symposium Series*, vol. **429**.



YOON, E., PARK, K., LEE, H., YANG, J.-H. & LEE, C. 2007. Estimation of excess Cancer Risk on Time-Weighted Lifetime Average Daily Intake of PAHs from Food Ingestion. *Human and Ecological Risk Assessment: An International Journal*, **13**, 669-680.