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SHORT COMMUNICATION / COMUNICACIÓN BREVE

A SIMPLE TOOL TO ASSESS THE EFFECT OF WATER-SOLUBLE SOIL POLLUTANTS ON ENZYME ACTIVITY IN HUMAN WHOLE BLOOD SAMPLES USING WST-1 ASSAY

Una herramienta sencilla para evaluar el efecto de los contaminantes del suelo solubles en agua sobre la actividad enzimática en muestras de sangre humana entera utilizando el ensayo WST-1

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Key words: hazardous compounds, water soil extracts, tetrazolium salts, environmental biomonitoring.

ABSTRACT

Hazardous compounds accumulated in soil affect soil quality. Here we show that WST-1 can be used for evaluation of the effect of water soil extracts on enzyme activity in human whole-blood samples. Soil samples from the Shumen Plateau Nature Park (NP) and the City Park in Shumen (SCP) were studied. We observed significant differences between control and treated whole-blood samples. These results indicated that soil water extracts affected normal metabolic activity. In conclusion, the WST-1 assay can be used for biomonitoring soil quality. The presence of harmful water-soluble compounds in tested soils may pose a risk to ecosystems and human health.

Palabras clave: compuestos peligrosos, extractos de agua y suelo, sales de tetrazolio, biomonitoreo ambiental.

RESUMEN

Los compuestos peligrosos acumulados en el suelo afectan la calidad del suelo. Aquí mostramos que WST-1 se puede utilizar para evaluar el efecto de los extractos de agua y suelo sobre la actividad enzimática en muestras de sangre entera humana. Se estudiaron muestras de suelo de Shumen Plateau Nature Park (NP) y City Park en Shumen (SCP). Observamos diferencias significativas entre las muestras de sangre entera tratadas y de control. Estos resultados indicaron que los extractos de agua del suelo afectaron la actividad metabólica normal. En conclusión, el ensayo WST-1 se puede utilizar para el biomonitoreo de la calidad del suelo. La presencia de compuestos solubles en agua nocivos en los suelos analizados puede representar un riesgo para los ecosistemas y la salud humana.

INTRODUCTION

Human impact is the main reason for changes in ecosystem services (MEA 2005). Hazardous compounds accumulated in soil affect soil quality. Soil pollution is studied using plant and animal test-systems. Humans also can serve as bioindicators in biomonitoring studies (Kamal and Malik 2017). In vitro experiments with blood samples are a promising approach to detect eco-health. As a body transport medium, blood reflects immediate exposure to changes in the environment (Garg 2017). For example, Furuyama et al. (2009) showed that ultrafine (20 nm) and fine (200 nm) particles are translocated from alveoli to the extrapulmonary organs via blood circulation and alveolar macrophages.

Soil can influence human health in different ways (Steffan et al. 2018). In vitro tests are able to detect many negative effects of pollutants. The WST-1 assay was successfully applied in biomonitoring studies using animal and plant test objects (Catala et al. 2009, Barriga-Vallejo et al. 2016). The assay principle is based upon the reduction of tetrazolium salts to formazan by dehydrogenases, leading to a colored complex whose quantity can be measured spectrophotometrically and is proportional to the number of viable cells. The reduction of the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) reagent are inside and outside the mitochondria and its solubility in the growth medium. This enzymatic activity is performed only by living cells according their metabolic activity (López et al. 2020). Exposure to toxic substances has a negative impact on the body metabolism. Tetrazolium salts are proved to be useful tools for measuring the metabolic activity of cells (Berridge et al. 2005, López et al. 2020). Previously we demonstrated the effectiveness of the WST-1 test to assess salt stress using whole blood samples from *Natrix tessellata*, Laurenti, 1768 (Koleva et al. 2017). Here we show that WST-1 can be used for evaluation of the effect of water soil extracts on enzyme activity in human whole-blood samples.

MATERIAL AND METHODS

The study areas are the Shumen Plateau Nature Park (NP) and the City Park in Shumen (SCP). We used the same soil samples than in our previous study (Koynova et al. 2017, Koleva et al. 2018). After preparing the soil for laboratory tests, unused soil was stored at –20 ºC. Before the tests, the soil was thawed. To avoid influence of mutagenic metabolic products of soil microorganisms, soil samples were sterilized by autoclaving. Samples of soil suspensions were prepared from one part soil and two parts culture media (Dulbecco's Modified Eagle Medium with gentamicin and penicillin). They were allowed to stand at room temperature for 24 h to prepare a soil extract of water-soluble compounds. The samples were centrifuged at 1000 g for 10 min. The soil extracts were filtered using a 0.22 μm filter. Ten percent fetal bovine serum was added to each extract.

The blood samples were provided by a young healthy donor. Each treatment sample contained 50 μL whole blood, 450 μL soil extracts, and 50 μL of WST-1 (Roche Applied Science). The control contained 50 μL whole blood, 450 μL complete culture media and 50 μL of WST-1. The samples were gently suspended and incubated for 4 h at 37 ± 1 ºC in 5 % CO_2 humidified incubator (Memmert, GmbH).

After treatment, 50 mL of the surface layer of each sample were carefully taken and placed in microplate well. The formazan quantity was measured spectrophotometrically at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The change in enzyme activity was calculated as a percentage of the absorbance of the sample from the absorbance of the control. The test was carried out in three replicates. Student's t-test was performed. The level of statistical significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

The Shumen Plateau Nature Park and Shumen city park (Bulgaria) are important green spaces for outdoor recreation (Koynova 2018). Soil quality is a key factor for ecosystem services. Exposure to contaminated soil poses a significant risk to human health (Kamal 2017). So, biomonitoring of soil pollution is vitally important.

In the present study, the influence of water soil extracts on enzyme activity in whole blood samples was evaluated. We observed significant differences between negative control and treated whole-blood samples (**Fig. 1**). The most notable was negative control samples from the peri-urban Nature Park (SNP). The ability of enzymes in whole-blood samples to reduce tetrazolium salt decreased from 2.9 to 6.6-fold in comparison with the control ($P \leq 0.05$). Strong inhibition was measured also after treatment with the sample from the city park (SCP): 3.1-fold in comparison with the negative control ($P \leq 0.05$). This data indicates that soil water extracts

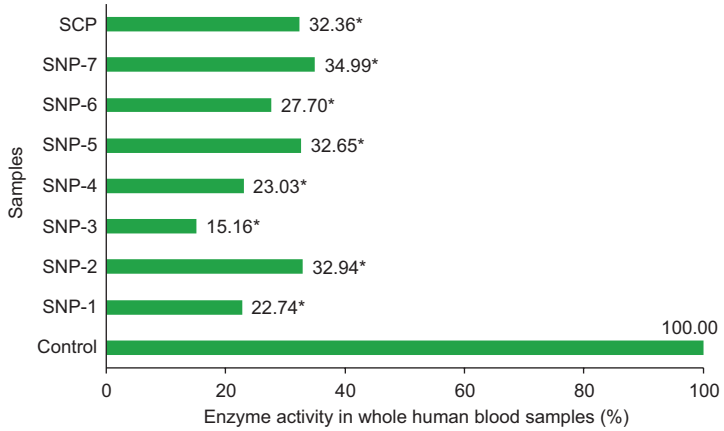

Fig. 1. Effect of water soil extracts on enzyme activity in whole human blood samples using WST-1 test. SCP: water extract of soil from City Park of Shumen; SNP: water extract of soil from Nature Park Shumen. *P ≤ 0.05.

affected normal metabolic activity. Other studies also reported the influence of toxic pollutants on enzymes (Christensen et al. 1982, Zolkipli-Cunningham and Falk 2017).

The present study shows that the WST-1 test can be used as a simple and useful tool to assess the presence of water-soluble pollutants in soil. Results from the WST-1 test are in accordance with our previous samples using *Allium cepa* (Koynova et al. 2017, Koleva et al. 2018). Other authors also have found a good correlation between tests with human/animal and plant test objects (Grant 1978, Fiskesjö 1993, Tedesco and Laughinghouse 2012, Palmieri et al. 2016, dos Reis et al. 2017).

CONCLUSION

The WST-1 assay can be used for biomonitoring soil quality. The study results show the presence of harmful water-soluble compounds in soils from green spaces for outdoor recreation in Nature Park Shumen Plateau and Shumen city park (Bulgaria). This observation revealed a risk for ecosystems and human health.

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REFERENCES

Barriga-Vallejo C., Aguilera C., Cruz J., Banda-Leal J., Lazcano D. and Mendoza R. (2017). Ecotoxicological biomarkers in multiple tissues of the *Neotenic ambystoma* spp. for a non-lethal monitoring of contaminant exposure in wildlife and captive populations. Water, Air, and Soil Pollution 228, 415. https://doi.org/10.1007/s11270-017-3590-3

Berridge M.V., Herst P.M. and Tan A.S. (2005). Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnology Annual Review 11, 127-152. https://doi.org/10.1016/S1387-2656(05)11004-7

Catala M., Esteban M., Rodríguez-Gil J.L. and Quintanilla L.G. (2009). Development of a naturally miniaturised testing method based on the mitochondrial activity of fern spores: A new higher plant bioassay. Chemosphere 77, 983-988. https://doi.org/10.1016/j.chemosphere.2009.07.080

Christensen G.M., Olson D. and Riedel B. (1982). Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. Environmental Research 29 (2), 247-255. https://doi.org/10.1016/0013-9351(82)90026-3

Dos Reis G.B., Andrade-Vieira L.F., Moraes I. de C., César P.H.S., Marcussi S. and Davide L.C. (2017). Reliability of plant root comet assay in comparison with human leukocyte comet assay for assessment environmental genotoxic agents. Ecotoxicology and Environmental Safety 142, 110-116. https://doi.org/10.1016/j.ecoenv.2017.04.004

Fiskesjö G. (1993). The *Allium* test in wastewater monitoring. Environmental Toxicology and Water Quality 8 (3), 291-298. https://doi.org/10.1002/tox.2530080306

Furuyama A., Kanno S., Kobayashi T. and Hirano S. (2009). Extrapulmonary translocation of intratracheally instilled fine and ultrafine particles via direct and alveolar macrophage-associated routes. Archives of Toxicology 83, 429-437. https://doi.org/10.1007/s00204-008-0371-1

Garg V. (2017). Histological features and studies of fish *Channa punctatus* treated with detergent. International Journal of Education and Science Research 4 (3), 70-73. https://www.ijesrr.org/publication/43/IJESRR%20V-4-3-10.pdf

Grant W.F. (1978). Chromosome aberrations in plants as a monitoring system. Environmental Health Perspectives 27, 37-43. https://doi.org/10.1289/ehp.782737

Kamal A. and Malik R.N. (2017). Human being as biomonitor of soil xenobiotics. In: Xenobiotics in the soil environment. Monitoring, toxicity and management. (Hashmi M.Z., Kumar V. and Varma A., Eds.). Springer International Publishing AG, Cham, Switzerland, 55-71.

Koleva V., Kornilev Y., Telenchev I., Lukanov S., Hristova B. and Natchev N. (2017). Salt tolerance's toll: Prolonged exposure to saline water inflicts damage to the blood cells of dice snakes (*Natrix tessellata*). Web Ecology 17, 1-7. https://doi.org/10.5194/we-17-1-2017

Koleva V., Dragoeva A., Koynova T. and Natchev N. (2018). Soil pollution screening using physicochemical and cytogenetic approaches: A case study of a Bulgarian suburban nature park. Polish Journal of Environmental Studies 27 (3), 1-8. https://doi.org/10.15244/pjoes/76409

Koynova, T., Koleva V., Dragoeva A. and Kuleva I. (2017). Cytotoxicity and genotoxicity of soil in Shumen city park. Proceedings. Central Bohemia University International Conference Innovations in Science and Education. Prague, Czech Republic, March 22-24, 1149-1153. https://doi.org/10.12955/cbup.v5.1086

Koynova T. (2018). Comparative analysis of Nature Park Shumen plateau and Shumen city park as green spaces. Acta Scientifica Naturalis 5 (2), 57-67.

López I., Balderas-Rentería I, Idalia Gómez, González-Santiago O., González-Barranco P., Garza-Ocañas L. and Ramírez-Cabrera M.A. (2020). MTT vs. WST-1, efficiency, cost, time, and waste generation: Evaluating the silver nanoparticles cytotoxicity. Revista de Ciencias Farmacéuticas y Biomedicina 3 (1), 3-12.

MEA (2005). Ecosystems and human well-being: Synthesis. Millennium Ecosystem Assessment. Report, Washington, DC, 155 pp.

Palmieri M.J., Andrade-Vieira L.F., Trento M.V.C., de Faria Eleutério M.W., Luber J., Davide L.C. and Marcussi S. (2016). Cytogenotoxic effects of Spent Pot Liner (SPL) and its main components on human leukocytes and meristematic cells of *Allium cepa*. Water, Air, and Soil Pollution 227 (5), 156.

Steffan J.J., Brevik E.C., Burgess L.C. and Cerdà A. (2018). The effect of soil on human health: An overview. European Journal of Soil Science 69 (1), 159-171. https://doi.org/10.1111/ejss.12451

Tedesco S.B. and Laughinghouse IV H.D. (2012). Bioindicator of genotoxicity: The *Allium cepa* test. In: Environmental contamination (Srivastava J.K., Ed.). IntechOpen, London, UK, 137-156.

Zolkipli-Cunningham Z. and Falk M.J. (2017). Clinical effects of chemical exposures on mitochondrial function. Toxicology 391, 90-99. https://doi.org/10.1016/j.tox.2017.07.009