Agriculture and Natural Resources 52 (2018) 366–370

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Conventional farming reduces the activity of earthworms: Assessment of genotoxicity test of soil and vermicast

Jaswinder Singh,[a, d, e, *] Sharanpreet Singh,[b] Adarsh Pal Vig,[b] Sartaj Ahmad Bhat,[b] Swarndeep Singh Hundal,[c] Rui Yin,[d, e] Martin Schädler[d, e]

[a] Department of Zoology, Khalsa College, Amritsar, Punjab, 143002, India
[b] Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, 143005, India
[c] Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab, 141004, India
[d] Department Community Ecology, Helmholtz - Centre for Environmental Research-UFZ, Halle, 06110, Germany
[e] German Center for Integrative Biodiversity Research (iDiv), Leipzig, 04103, Germany

ARTICLE INFO

Article history:
Received 1 May 2017
Accepted 1 August 2017
Available online 24 October 2018

Keywords:
Allium cepa
Chromosomal aberrations
Earthworms
Genotoxicity test
Vermicast

ABSTRACT

The activity of earthworms in an agricultural field and botanical garden was substantiated. Samples of vermicast produced by earthworms along with the soil were collected from an agricultural field (conventional farm) and a botanical garden and their genotoxic assessment were observed on the root tips cells of *Allium cepa*. The mitotic index (MI) was increased in both vermicast samples compared to soil. However, the mean (±SE) MI was greater in the vermicast (13.93 ± 0.67) collected from the botanical garden compared to the agricultural vermicast (11.40 ± 0.51). Chromosomal aberration levels were greater in the soil of the agricultural field (14.88%) compared to soil from the botanical garden (9.11%), whereas a reduction in chromosomal aberrations was observed in the vermicast of both sites. The number of earthworms was less on the conventional farm (18 earthworms/1800 cm^2 area) compared to the botanical garden (72 earthworms/1800 cm^2 area). Thus, this study showed that the activity of earthworms was less in an agricultural field where there may have been more application of chemical fertilizers and pesticides compared to a site using more organic manure with reduced genotoxicity.

Introduction

Sustainable agriculture is the production of food from plant or animals by using different agricultural techniques that protect public health, human communities, the environment and animal welfare (Datta et al., 2016). During the green revolution, an extensive quantity of chemical pesticides and fertilizers were used to boost the crop yield from agricultural land but during the last four decades, the quality of the soil has reduced (Gupta et al., 2014; Vanita et al., 2014; Datta et al., 2016).

A sustainable solution to overcome the hazardous effects of modernized agriculture is to develop a farming system which is economically productive and long-lasting by simple and inexpensive practices involving the introduction of earthworms. Earthworms have the ability to digest and convert low quality organic matter into a nutrient rich product by developing a mutualistic relationship with soil microflora (Singh et al., 2014). The feeding and casting activities of earthworms deposit organic remains into the soil and this activity also enhances nutrient cycling and improves soil structural development (Datta et al., 2016; Singh et al., 2016a). The vermicast egested by earthworms is more stable than the soil and also contains certain inorganic and organic materials, enzymes, microorganisms and plant growth hormones acquired during the passage of soil aggregates through the gut of the earthworm (Tersic and Gosar, 2012). The cast egested by earthworms also improves the physicochemical characteristics of the soil (Chaoui et al., 2003). In addition, earthworm cast also increases plant dry weight, plant nitrogen uptake and provides a better root growth medium by improving soil porosity (Scott et al., 1996). Agricultural soils are increasingly becoming contaminated by anthropogenic activities and this can cause severe health risk to humans. For example, Lagerlof et al. (2002) reported that the population density of earthworms was lower and less diverse in arable soil compared with uncultivated habitats. The *Allium cepa* test was also used to detect the presence of harmful pollutants in

* Corresponding author. Department of Zoology, Khalsa College, Amritsar, Punjab, 143002, India.
E-mail address: singhjassi75@yahoo.co.in (J. Singh).

https://doi.org/10.1016/j.anres.2018.10.012

soil and water (Leme and Marin-Morales, 2009). This sensitive test is an alert system to assess the genotoxic potential of soil and other industrial wastes (Ansari and Malik, 2009; de Souza Pohren et al., 2013; Bhat et al., 2015).

In a previous study, Singh et al. (2016a) compared the physicochemical properties of vermicast and soil from an agricultural field and a botanical garden and also reported the effect of different agricultural management practices such as ploughing and the application of chemical fertilizers and pesticides on the activity of earthworms. The botanical garden and agricultural field were chosen for the study because in the botanical garden only organic manure was used for cultivation of flowering plants while conventional farming methods used chemical fertilizers and pesticides extensively in the agriculture field. In the present study, a genotoxicity assessment test was applied to the same samples of vermicast and soil. The objectives of the present study were: 1) to investigate the effect of soil and vermicast on the mitotic index (MI) of the root tips cells of *Allium cepa*; 2) to study chromosomal aberrations in the root tip cells of *Allium cepa* exposed to soil and vermicast; and 3) to study the activity of earthworms with regard to genotoxicity reduction.

Materials and methods

Study site, sampling of soil and vermicast

The soil and vermicast samples were collected in duplicate from the botanical garden (N 31^0 63′ 22.3″, E 074^0 87′ 15.2″) at Khalsa College, Amritsar, India and from a conventional farm (N 31^0 56′ 53.3″, E 074^0 59′ 29.2″), Talwandi Rama village, Gurdaspur, India. The coordinates were measured using a GPS system (Garmin 78) (Singh et al., 2016a). The activity of earthworms is usually higher at night and therefore vermicast was collected from the upper surface of the soil early in the morning using a brush. Earthworm sampling was also done using a hand sorting and digging method with a quadrate of size 30 cm × 30 cm (1800 cm^2). The soil and vermicast samples were air-dried and stored in polythene bags. The physicochemical properties of soil and vermicast samples from the same sites have already been reported by Singh et al. (2016a).

Allium cepa test

The *Allium cepa* root chromosomal aberration test (*in situ* treatment) was used to investigate the genotoxic potential of soil and vermicast samples from the agricultural field and botanical garden (Sharma and Sharma, 1980). The onion (*Allium cepa*) bulbs were purchased from a local market. The bulbs were denuded and scraped at the bottom to expose the root primordial. The denuded onion bulbs were allowed to root directly for 48 h in soil and vermicast samples contained in treatment glass jars (26 mm height × 90 mm diameter) in triplicate (three onion bulbs for each sample) at room temperature. Sand was used as a negative control. After treatment, the root tips were washed, plucked and fixed in Farmer's fluid (formed by mixing glacial acetic acid and ethanol with a ratio of 1:3, respectively) for 24 h and then stored at 4 °C. The slides of root tips were prepared by first hydrolyzing in 1N HCl for 1 min and then transferring to a watch glass containing a mixture of 1N HCl and aceto-orecin (1:9, respectively) and heating intermittently for 2–3 min. After 25–30 min, the root tips were squashed in a drop of 45% glacial acetic acid on a slide, covered and sealed with dibutyl phthalate and xylene (DPX) solution. The slides were labeled, scored and observed for different types of chromosomal aberrations under a trinocular microscope (Olympus). About 450 dividing cells were scored per sample. The photographs were taken with the help of MIPS (Multiband Imaging Photometer System) camera fixed on the microscope. The mitotic index (MI) was calculated using Equation (1):

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100 \quad (1)$$

Statistical analysis

The mitotic index data were presented as mean ± SE of triplicates. One-way analysis of variance (ANOVA) was applied to compare the MI in both soil and vermicast samples. The chromosomal aberrations of soil and vermicast samples were presented as percentages and the level of significance was determined using a chi-square test. Statistical analysis was done with the help of Minitab software program (version 14; Chicago, USA).

Results and discussion

Singh et al. (2016a) analyzed the physicochemical properties of earthworm cast from an agricultural field and botanical garden and compared the results with soils samples from the respective sites. Study reported that pH, Total dissolved solids (TDS), EC, Ca, Na, Li, K, and heavy metals were lower in the vermicast while organic carbon (OC), P and N was more in the vermicast compared to the respective soil. The effects of soil and vermicast of both conventional farm and botanical garden on the MI of *A. cepa* root tip cells are shown in Fig. 1. The MI increased significantly ($p < 0.05$) in both vermicast samples compared to the respective soil samples. The percentage increase in the MI in the botanical garden vermicast was 13.93 ± 0.67 compared to the botanical garden soil (10.51 ± 0.51) while the percentage increase of MI in agricultural vermicast was 11.40 ± 0.51 compared to soil from the agricultural field (8.43 ± 1.04). The number of earthworms was also less in the agricultural field under conventional farming (18 earthworms/1800 cm^2 area) compared to the botanical garden using organic manure (72 earthworms/1800 cm^2 area). Castellanos-Navarrete et al. (2012) and Singh et al. (2016b) also reported that different agricultural management practices affected the population density and biomass of earthworms, with effects on cast production. Pelosi et al. (2015) also observed that earthworm communities (abundance and biomass) increased in organic farms compared to conventional farms in two sampling periods. This high abundance and biomass of earthworms in the soil maintain aerobic conditions which not only favor degradation of contaminants but also facilitate the microbial and biochemical activities in the soil (Datta et al., 2016). Campani et al. (2017) also considered earthworms as a biomarker for sustainable agriculture because they represent a strong means of investigating the toxicity of agricultural land and of identifying practices and products with low environmental input. The vermicast egested by earthworms in the field is an ideal organic fertilizer which is required by plants for their growth and prevents them from harmful insect pests without polluting the environment (Joshi et al., 2014). Suthar (2009) also reported that plants with vermicompost treatment had higher values of various growth and yield parameters compared to the values of these parameters from a chemical fertilizer treatment.

The MI and levels of chromosome aberration are considered as indicators of the genotoxic potential of various environmental pollutants (de Souza Pohren et al., 2013). The cytotoxicity potential of any chemical or soil can be determined by the increase or decrease in the MI compared to the control (Radic et al., 2010). Interference in the normal cell cycle and impaired cell proliferation result in a reduction in the MI (Bhat et al., 2015). The results of the present

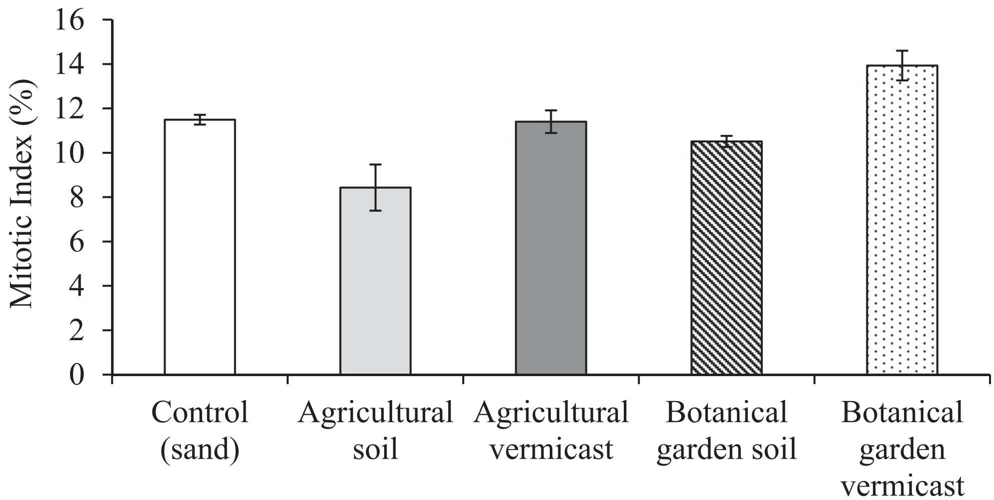

Fig. 1. Effect of soil and vermicast on the mitotic index of the root tip cells of *Allium cepa* (where error bars indicates ± S.E.).

study revealed a reduction in the MI and an increase in chromosome aberrations of both soil samples from the agricultural field and the botanical garden with respect to the control. The MI was reduced more in agricultural soil compared to botanical garden soil which may have been due to a higher application of chemical fertilizers and pesticides in the agricultural field. The frequencies of different types of chromosome aberrations in the soil and vermicast samples of conventional farm and botanical garden are given in Table 1. The chromosome aberrations were apportioned into physiological aberrations (delayed anaphase, c-mitosis, laggards, vagrants, stickiness) attributable to spindle abnormalities and clastogenic aberrations (chromosomal bridges, chromosome breaks) attributable to direct action on chromosomes (Fig. 2). The soil samples from both the agricultural field and botanical garden induced significant chromosomal aberrations ($p < 0.01$), whereas vermicast significantly ($p < 0.05$) produced minimum chromosome aberrations compared to the respective controls. The agricultural soil produced the highest level of chromosomal abnormalities (14.88%), while the level of chromosomal abnormalities in the garden soil was 9.11% (Table 1). Vermicast collected from the botanical garden had the fewest (6.68%) aberrations while vermicast from the agricultural field had 8.0% aberrations. The level of c-mitosis was higher in the agricultural soil than the control and other samples. Delayed anaphase and c-mitosis were the most detected aberrations followed by chromatin bridges. The high numbers of physiological aberrations (55) and clastogenic aberrations (12) in the agricultural soil indicated that the agriculture soil had more genotoxic effects compared to the botanical garden soil which could have been due to the extensive use of pesticides and chemical fertilizers in the agricultural field. Other researchers have also reported higher chromosome aberrations in agricultural soils (Dragoeva et al., 2009; Masood and Malik, 2013; Souza et al., 2013).

The vermicast samples from both the agricultural field and botanical garden confirmed the genotoxicity reduction compared to agriculture and botanical garden soil respectively. Genotoxicity reduction by earthworms was higher in the botanical garden than of the agricultural land because of the greater abundance of earthworms in the botanical garden soil compared to the agriculture land. The earthworms were more active in the botanical garden than the agricultural field. The reduced activity of earthworms in the agricultural field may have been due to different agricultural practices and the extensive use of pesticides and chemical fertilizers. Thus, the feeding and casting activities of earthworms not only increased the nutrient content of soil but also reduced the toxicity which indicates that earthworms have a potential role in sustainable agriculture. Thus, the feeding and casting activity of the earthworms stabilized the soil and modified the physicochemical nature of the soil, which was beneficial for the growth of the plants.

Table 1
Chromosomal aberrations in the root tip cells of *Allium cepa* exposed to soil and vermicast.

Type of chromosomal aberration	Sand (control)	Agricultural soil	Agricultural vermicast	Botanical garden soil	Botanical garden vermicast
	Number of aberrant cells[a]	Number of aberrant cells[a]	Number of aberrant cells[a]	Number of aberrant cells[a]	Number of aberrant cells[a]
Physiological aberrations (PA)					
c-Mitosis	2	20	6	6	7
Delayed anaphase	3	21	17	20	16
Laggard/s	–	2	–	–	–
Stickiness	–	6	2	4	1
Vagrant/s	–	6	1	2	2
Total PA	5	55	26	32	26
Clastogenic aberrations (CA)					
Chromatin bridge/s	1	11	10	9	5
Chromosomal break/s	–	1	–	–	–
Total CA	1	12	10	9	5
Total aberrant cells (PA + CA)	6	67	36	41	31
Aberration (%)	1.33	14.88**	8.0*	9.11**	6.88

Significance level was determined using a chi square test, $* = p < 0.05$, $**p < 0.01$.
[a] Data obtained from 450 cells.

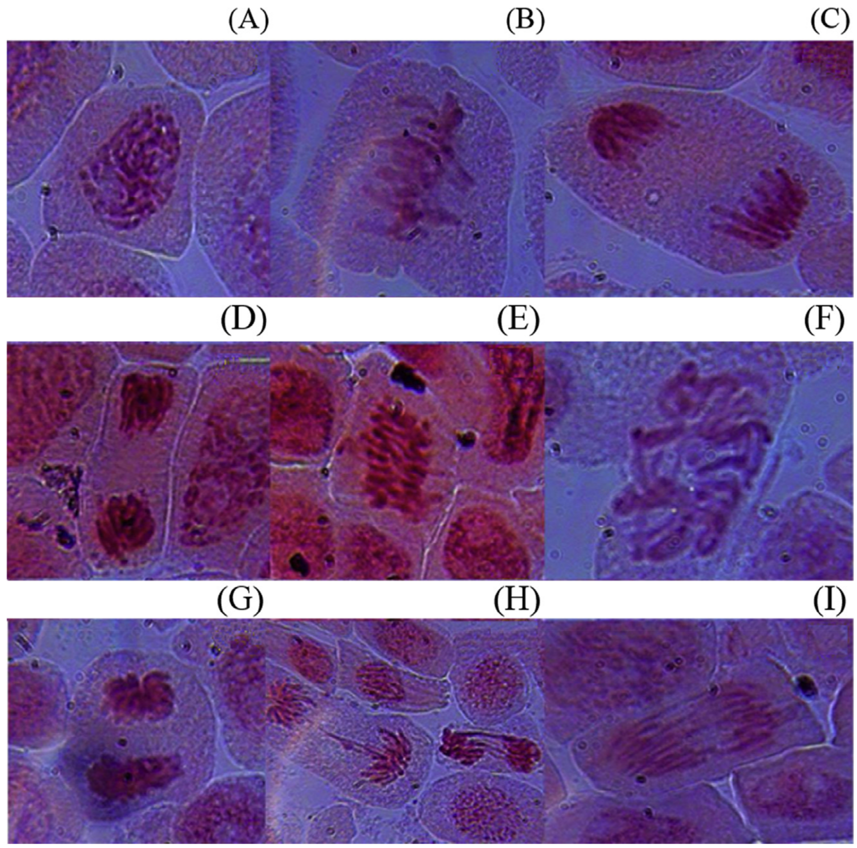

Fig. 2. Root tip cells of *Allium cepa* showing normal stages: (A) prophase; (B) metaphase; (C) anaphase; (D) telophase. Chromosomal aberrations: (E) delayed anaphase; (F) c-mitosis; (G) stickiness; (H) bridge; (I) multiple bridges.

The current results corroborate Bhat et al. (2015) who reported that earthworms reduced the toxicity of wastes and had the ability to detoxify contaminated soil. The increase in the MI and the decrease in chromosomal aberrations in the vermicast samples of both the agricultural field and botanical garden indicated that the earthworms had the ability to reduce the soil toxicity. Farmers must be educated and made aware of the potential impact of using insecticides and pesticides in agricultural fields so that such use does not harm the earthworm species. Earthworm-friendly agriculture practices should be developed to maintain their population in the soil for long-term soil productivity (Rajkhowa et al., 2015). The presence of earthworms in soil improved the physicochemical structure of the soil by increasing soil porosity and maintaining the physical, chemical and biological properties of soil (Datta et al., 2016) and thus plays an important role in sustainable agriculture.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

The authors thank the Science and Engineering Research Board (SERB) Department of Science and Technology, GOI, New Delhi, India for financial assistance in the form of a Major Research Project Letter No SR/SO/AS-030/2013. The corresponding author also acknowledges the Alexander Humboldt Foundation, Germany.

References

Ansari, M., Malik, A., 2009. Genotoxicity of agricultural soils in the vicinity of industrial area. Mutat. Res. 673, 124–132.
Bhat, S.A., Singh, J., Vig, A.P., 2015. Vermistabilization of sugar beet (*Beta vulgaris* L.) waste produced from sugar factory using earthworm *Eisenia fetida*: genotoxic assessment by *Allium cepa* test. Environ. Sci. Pollut. Res. 22, 11236–11254.
Campani, T., Caliani, I., Pozzuoli, C., Romi, M., Fossi, M.C., Casini, S., 2017. Assessment of toxicological effects of raw and bioremediated olive mill waste in the earthworm *Eisenia fetida*: a biomarker approach for sustainable agriculture. Appl. Soil Ecol. 119, 18–25.
Castellanos-Navarrete, A., Rodrıguez-Aragones, C., de-Goede, R.G.M., Kooistra, M.J., Sayre, K.D., Brussaard, L., Pulleman, M.M., 2012. Earthworm activity and soil structural changes under conservation agriculture in central Mexico. Soil Till. Res. 123, 61–70.
Chaoui, H.I., Zibilske, L.M., Ohno, T., 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biol. Biochem. 35, 295–302.
Datta, S., Singh, J., Singh, S., Singh, J., 2016. Earthworm, pesticides and sustainable agriculture: a review. Environ. Sci. Pollut. Res. 23, 8227–8243.
de Souza Pohren, R., da Costa, T.C., Vargas, V.M.F., 2013. Investigation of sensitivity of the *Allium cepa* test as an alert system to evaluate the genotoxic potential of soil contaminated by heavy metals. Water Air Soil Pollut. 224, 1460. https://doi.org/10.1007/s11270-013-1460-1.
Dragoeva, A., Kalchevai, V., Slanev, S., 2009. Genotoxicity of agricultural soils after one year of conversion period and under conventional agriculture. J. Appl. Sci. Environ. Manag. 13, 81–83.
Gupta, S., Kushwah, T., Yadav, S., 2014. Role of earthworm in promoting sustainable agriculture in India. Int. J. Curr. Microbio. Appl. Sci. 3, 449–460.
Joshi, R., Singh, J., Vig, A.P., 2014. Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. Rev. Environ. Sci. Biotechnol. 14, 137–159.
Lagerlof, J., Goffre, B., Vincent, C., 2002. The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agric. Ecosyst. Environ. 89, 91–103.
Leme, D.M., Marin-Morales, M.A., 2009. *Allium cepa* test in environmental monitoring: a review on its application. Mutat. Res. 682, 71–81.
Masood, F., Malik, A., 2013. Cytotoxic and genotoxic potential of tannery waste contaminated soils. Sci. Total Environ. 444, 153–160.

Pelosi, C., Bertrand, M., Thenard, J., Mougin, C., 2015. Earthworms in a 15 years agricultural trial. Appl. Soil Ecol. 88, 1–8.
Radic, S., Stipanicev, D., Vujcic, M.M., Sirac, S., Pevalek-Kozlina, B., 2010. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Sci. Total Environ. 408, 1228–1233.
Rajkhowa, D.J., Bhattacharyya, P.N., Sarma, A.K., Mahanta, K., 2015. Diversity and distribution of earthworms in different soil habitats of Assam, North-East India, an Indo-Burma biodiversity hotspots. In: Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences, vol. 85, pp. 389–396. India.
Scott, N.A., Cole, C.V., Elliott, E.T., Huffman, S.A., 1996. Soil textural control on decomposition and soil organic matter dynamics. Soil Sci. Soc. Am. J. 60, 1102–1109.
Sharma, A.K., Sharma, A., 1980. Chromosome Techniques: Theory and Practice. Butterworths, London, UK.
Singh, J., Kaur, A., Vig, A.P., 2014. Bioremediation of distillery sludge into soil-enriching material through vermicomposting with the help of *Eisenia fetida*. Appl. Biochem. Biotech. 174, 1403–1419.
Singh, S., Singh, J., Vig, A.P., 2016a. Earthworm as ecological engineers to change the physico-chemical properties of soil: soil *vs* vermicast. Ecol. Eng. 90, 1–5.
Singh, S., Singh, J., Vig, A.P., 2016b. Effect of abiotic factors on distribution of earthworms in different land use patterns. J. Basic Appl. Zool. 74, 41–50.
Souza, T.S., Hencklein, F.A., Angelis, D.F., Fontanetti, C.S., 2013. Clastogenicity of land farming soil treated with sugar cane vinasse. Environ. Monit. Assess. 185, 1627–1636.
Suthar, S., 2009. Impact of vermicompost and composted farmyard manure on growth and yield of garlic (*Allium stivum* L.) field crop. Int. J. Plant Prod. 3, 27–38.
Tersic, T., Gosar, M., 2012. Comparison of elemental contents in earthworm cast and soil from a mercury contaminated site (Idrija area, Slovenia). Sci. Total Environ. 430, 28–33.
Vanita, C., Piar, C., Avinash, N., Kaur, J.K., Yogesh, P., 2014. Evaluation of heavy metals contamination and its genotoxicity in agricultural soil of Amritsar, Punjab, India. Int. J. Res. Chem. Environ. 4, 20–28.