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The impact of atrazine on several biochemical properties of chernozem soil

LJ. RADIVOJEVIĆ, S. GAŠIĆ*, LJ. ŠANTRIĆ and R. STANKOVIĆ-KALEZIĆ

Institute of Pesticides and Environmental Protection, Banatska 31b, P.O. Box 163, 11080 Belgrade, Serbia

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Abstract: The impact of the pesticide atrazine on biochemical processes in soil was investigated. Atrazine loadings of 8.0, 40.0 and 80.0 mg/kg soil were laboratory tested in an experiment set up on a clay loam soil. Dehydrogenase activity, change in biomass carbon, soil respiration and metabolic coefficient were examined. The samples were collected for analysis 1, 7, 14, 21, 30 and 60 days after atrazine application. The acquired data indicated that the effect of atrazine on the biochemical activity of the soil depended on its application rate and duration of activity, and the effect was either stimulating or inhibiting. However, the detected changes were found to be transient, indicating that there is no real risk of the compound disrupting the balance of biochemical processes in soil.

Keywords: atrazine; soil; dehydrogenase; biomass carbon; respiration.

INTRODUCTION

Ever since they were first discovered, pesticides have become an indispensable segment of sound agriculture. Their development and production have soared tremendously and their application rates have increased steadily, so that warning reports of their presence in the environment have become increasingly frequent. Alternatives have therefore been sought since the 1980s and novel and high-safety compounds have been introduced that are not only applied at far lower levels but have more favorable ecotoxicological characteristics as well, so that environmental contamination can, to some extent, be kept under control.[1,2]

Atrazine, which was first introduced to the market in 1952, belongs to a group of pesticides that are moderately persistent and moderately mobile in soil. The half-life of atrazine varies between several days and several months.[3] As the compound has been on the market for a number of years and rather high amounts were applied over wide areas, atrazine residues have been detected both in surface and underground waters.[4,5] Domestic studies detected 1.0–4.13 μg L^{-1} of

* Corresponding author. E-mail: slavica.gasic@yahoo.com
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atrazine in surface waters and up to 0.3 μg L^{-1} in underground waters.[6] Its residues in surface and underground waters are consequently causing serious concern for human and animal health and the environment.[4,5,7]

As a segment of the environment, soil is a complex and dynamic multiphase system with the lithosphere, biosphere, hydrosphere and atmosphere in a perpetual state of balance. Various exogenous factors, primarily heavy metals, pesticides, fertilizers and many others, may disrupt and deteriorate the harmonious state within an ecosystem.[8] Growing concern for preserving soil as a natural resource have prompted researchers over the past years to focus on studying the changes occurring in soil or those that may occur in the future under certain harmful environmental factors, pesticides being an important one.[9–11] Various indicators may be used in such research, the most frequently employed being soil enzymes, biomass, respiration, *etc*.

This study is a follow-up to experiments conducted over the past few years in which atrazine residues were examined in Serbian agricultural soils. As atrazine residues were detected in all studied soil samples,[6] it was assumed that the pesticide and its metabolites may cause certain changes in soil, and in its wider ecosystem, and that experimental data could help assess the risk of disruption of the existing natural balance.

EXPERIMENTAL

The pesticide (herbicide) atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) tested in the experiments was a technical grade product of Agan Chemical Manufacturers, Ashdod, Israel. The application loads were: 8.0, 40.0 and 80.0 mg/kg soil. The lowest concentration tested was the recommended application rate (8 mg/kg), and the other two were five and ten times higher doses than that recommended. The experiments were performed using a chernozem soil of a clay loam texture (pH 7.10, organic matter 3.32 %, sand 21 %, silt 49 %, and clay 30 %) at Zemun Polje, Belgrade, Serbia. The soil chosen for the study had never been treated with pesticide before. Various management practices would otherwise have affected the microbial populations of the soil. In this way, it was possible to control the effects of a specific pesticide (atrazine).

Dehydrogenase activity, microbiological biomass carbon and soil respiration were examined as relevant biochemical indicators.[12-14]

Soil samples were collected from the upper layer (0–10 cm), carefully dried, sieved to pass through a 5 mm mesh and stored at 4 °C. Before use, the soils were air-dried at room temperature for 24 h. Each herbicide concentration was pipetted on to the surface of 1 kg of soil before homogenization on a rotating stirrer for 30 min. After homogenization, the soil was portioned into pots. Untreated soil served as the control. The experiments were conducted with four replications. The pots were kept in a controlled-environment chamber at 20±2 °C, 50 % air humidity and a 12–12 h day–night photoperiod throughout the experiment. The soil humidity was maintained at 50 % field capacity. Samples were collected for analysis 1, 7, 14, 21, 30 and 60 days after atrazine application.

The activity of the enzyme dehydrogenase was determined according to Tabatabai.[15] The soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) under

moist conditions at 37 °C for 24 h. Triphenylformazan (TPF), which is derived from triphenyltetrazolium chloride (TTC) as a product of enzyme activity, was determined spectophotometrically. Measurements were performed at 485 nm (Gilford stasar III, Model 2400) and the enzyme activity given as μg TPF/g soil.

Fumigation–extraction[16] was employed to determine microbiological biomass carbon. The samples were fumigated with non-alcohol-containing $CHCl_3$ under moist conditions for 24 h. After incubation, carbon was extracted with a 0.50 M solution of potassium sulfate and its content determined by titration with a 0.033 M solution of Mohr salt ($(NH_4)_2Fe(SO_4)_2$) in the presence of phenylanthranilic acid as the indicator. Non-fumigated samples were extracted under the same conditions. The microbiological biomass carbon was calculated based on the difference between carbon in the fumigated and non-fumigated samples using a factor 0.38.[17] The results are presented in μg C/g soil.

The Walter method[18] was employed to determine the soil respiration. The soil samples were incubated with sodium hydroxide under moist conditions at room temperature for 24 h. The carbon dioxide released during soil respiration was absorbed by a 0.10 M solution of sodium hydroxide, and the CO_2 content determined by titration with 0.10 M hydrochloric acid in the presence of an appropriate indicator (phenolphthalein or methyl orange). The results are presented in μg CO_2/g soil.

The microbiological metabolic coefficient, $q(CO_2)$, was computed from the ratio of the soil respiration intensity and the microbiological biomass.[19]

Statistical data processing was performed using PC Anova software. The F-test was applied to all variables and their interactions and, in case of a significant result, the LSD test was applied in individual comparisons. The probability levels 0.05 and 0.01 were used as signifycance criteria.

RESULTS AND DISCUSSION

Dehydrogenases are soil enzymes catalyzing degradation of organic matter in what is fundamentally a redox process. Soil dehydrogenases are predominantly microbiological in origin and their activities depend on the conditions within the soil ecosystem. Thus a higher enzyme activity indicates a greater intensity of mineralization of the organic matter.[15,20,21] The results of this experiment show a decreased activity of dehydrogenase for all applied atrazine concentrations from the 1st to the 30th day. The decrease ranged from 12.5–18.2 %, 4.8–24.8 % and 6.6–39.6 % for atrazine loadings of 8.0, 40.0 and 80.0 mg/kg soil (Fig. 1), respectively, and the differences found were statistically significant ($P < 0.01$). A lower enzyme activity indicates reduced redox intensity in the soil and the reduction degree depended on the concentration and duration of atrazine activity. The enzyme activity was reduced by the harmful activity of atrazine. As pesticides are to some degree environmental toxicants, their noxious activity may be described being manifested mostly through a disturbance of the natural balance. Dehydrogenases, which are free soil enzymes, are very susceptible to unfavorable factors originating from the surrounding environment, including pesticides, *i.e.*, to atrazine in this particular case. There was an increase of enzyme activity from the 30th to the 60th day and the values for treated and untreated soils were

similar at the end of the examination period, except for the highest concentration (80 mg/kg soil), where the value was lower (7.9 %). The obtained experimental data are consistent with the results reported by other authors on the effect of different pesticides on this enzyme.[22–27]

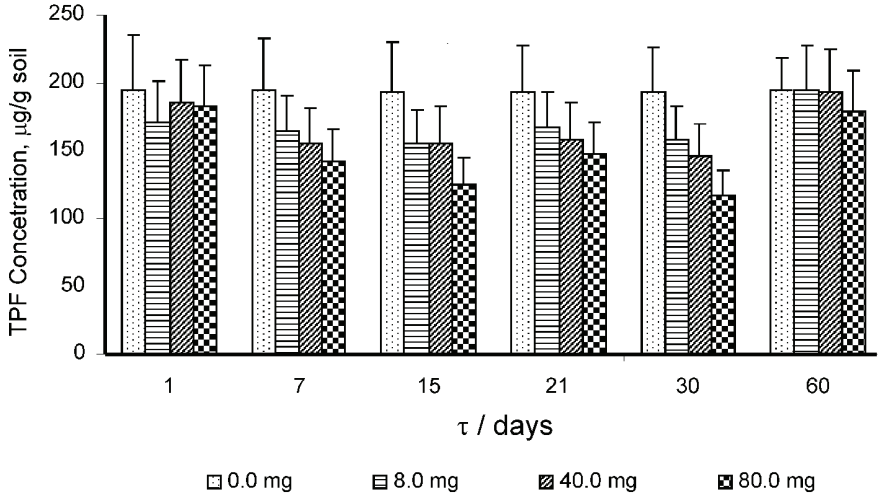

Fig 1. Effect of atrazine on dehydrogenase.

Data showing the effect of atrazine on the biomass carbon are presented in Fig. 2. The highest biomass carbon (1818 μg C/g soil) was found for an atrazine loading of 8.0 mg/kg soil (7 days after application) and the lowest (986 μg C/g soil) for an atrazine loading of 80.0 mg/kg soil (7 days after application). Reduced biomass carbon under concentrations of 40.0 and 80.0 mg/kg soil was recorded as early as one day after application and the inhibitory effect of the highest concentration (80.0 mg/kg soil) extended 21 days after application. Significant ($P < 0.01$) increases in the biomass carbon (2.1–45.6 %) were recorded under the lower concentrations of atrazine (8.0 and 40.0 mg/kg soil) from the 7th to the 30th day and at the end of the experiment the increases were 2.1 and 33.3 %, respectively, (Fig. 2). Wardle and Parkinson[28] assumed that a most dramatic decrease in the biomass carbon would occur immediately after pesticide application, when the concentration of compound in the soil solution is highest. A number of authors believe[29–32] that biomass carbon later grows primarily due to the restored populations of live organisms which have adapted to the particular pesticide present in the soil. Hence, a new biomass is formed which is metabolically very active and participates in various biochemical processes in the soil. The biomass carbon is also important for the rate of atrazine degradation in soil. Entry *et al.*[32] monitored the relationship between the degradation rate of atrazine and the microbiological biomass in young and old forests and detected an evident correlation, *i.e.*, that atrazine degraded faster in the older forests with a higher microbiological biomass.

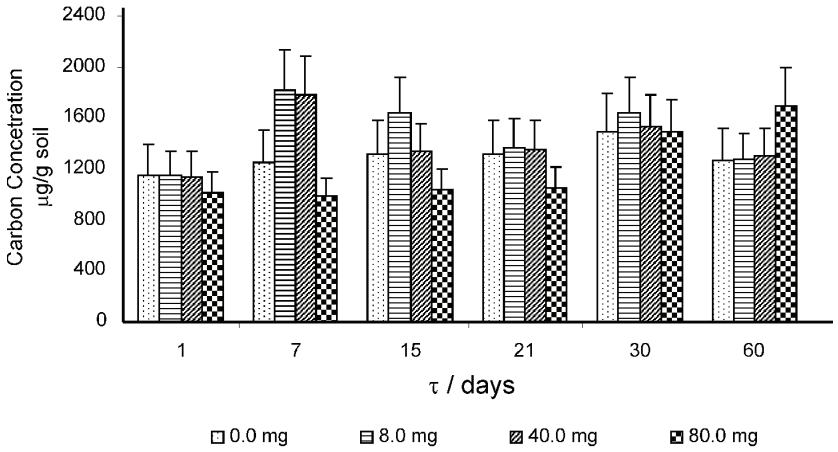

Fig 2. Effect of atrazine on microbiological biomass carbon.

There have also been other reports on the activity of different pesticides in relation to biomass carbon. For example, Perucci and Sacrponi[10,33] found that the effect of rimsulfuron and imazethapyr on biomass carbon depended on the soil moisture. Under reduced moisture, the harmful activity of rimsulfuron lasted 36 h but as long as 72 h under high moisture. Similar findings were also reported by Wardele and Parkinson,[28] as well as by Rath,[34] in their experiments investigating 2,4-D and glyphosate. Finally, Startton and Stewart[35] recorded harmful effects of glyphosate on soil biomass and respiration in Canadian coniferous forests, while 2,4-D has no effect.

The effect of atrazine on soil respiration depended primarily on pesticide concentration (Fig. 3). The respiration intensity ranged between 3.2 (80.0 mg/kg soil, 1 and 7 days after application) and 6.2 μg CO_2/g soil (40.0 mg/kg soil, 21 days after application). Under the highest concentration of atrazine (80.0 mg/kg soil), the respiration was reduced 13.3–31.8 % from the 1st until the 21st day. Between the 7th and 30th day, an increase in respiration (8.3–56.4 %) was observed under the applied concentrations of 8.0 and 40.0 mg/kg soil. At the end of the experiment (60 days after application), a significant decrease in respiration was detected for all the investigated concentrations. All the found differences were statistically significant ($P < 0.01$). Literature data show that atrazine, as well as other pesticides, cause a significant inhibition of soil respiration.[33] Some authors[36–38] found that herbicides from the group of substituted ureas increase soil respiration intensity four weeks after application. The authors believe that increased respiration intensity occurs due to increased activity of microorganisms populating the soil that are able to use the available carbon from the atrazine molecules for their physiological needs. This assumption was later experimentally confirmed by other authors as well.[39] Wardle and Parkinson,[28] Fleibach and Mader,[40] and Harden[29] found that other pesticides, such as 2,4-D and benomyl, in-

creased the intensity of respiration, while glyphosate and dinoseb had no effect on the process. However, other researchers reported that butachlor, alachlor, metolachlor and metribuzin reduced the intensity of this process.[41–43]

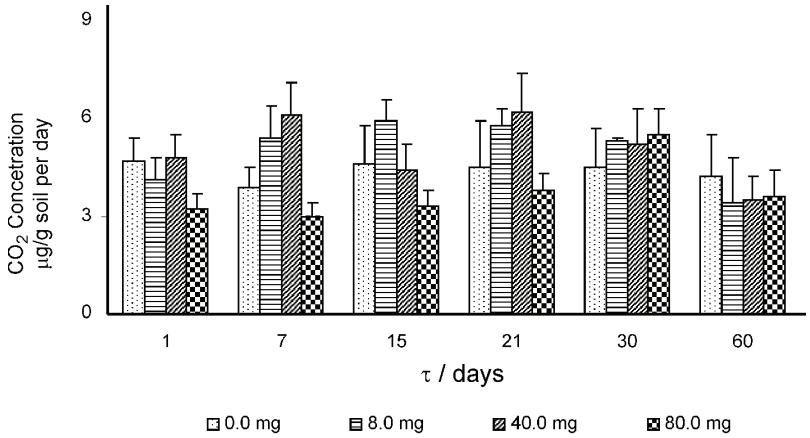

Fig 3. Effect of atrazine on soil respiration.

The metabolic coefficient, $q(CO_2)$, is the ratio between soil respiration and biomass carbon,[44] and a high value indicates an unfavorable environmental factor (*e.g.*, drought, soil cultivation, heavy metals, pesticides, *etc*.).[39] In this investtigation, increased values of the metabolic coefficient were recorded on the 1st (40.0 mg/kg soil), 7th (40.0 and 80.0 mg/kg soil), 15th (80.0 mg/kg soil), 21st (all examined concentrations) and 30th (40.0 mg/kg soil) day of the experiment. Reduced coefficient values were recorded after the 1st (80.0 mg/kg soil) and 60th day (all employed concentrations). All the detected differences were statistically significant ($P < 0.01$ and $P < 0.05$) (Fig. 4).

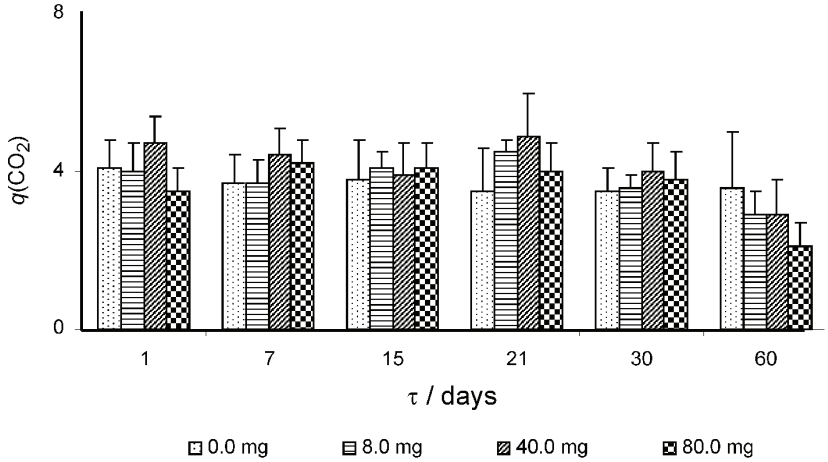

Fig 4. Metabolic coefficient variation under the effect of atrazine.

Multiple increases (1.7-, 3- and 5-fold) in the values of the metabolic coefficient have been reported in experiments performed on acid soils,[45] during drought[46] or after pesticide treatments. Concerning heavy metals, high coefficient values indicate a change in the energy flow in the basic metabolic processes, and that more carbon is required for their normal continuation.[46] Wardle *et al.*[28] also found high values of the $q(CO_2)$ coefficient 21 days after glyphosate and dinoseb treatments. Higher levels of this coefficient have been recorded in dinoseb trials, which were to be expected because the pesticide is more toxic than glyphosate. Jones and Ananye[39] found that propachlor caused no significant change in the coefficient, while it increased 20–25 % after metalaxyl application, depending on the environmental conditions. Earlier research by these authors showed more harmful effects of metalaxyl in dry than in moist soils, *i.e.*, under conditions less favorable for biosphere development. In the present experiments, increased values of the coefficient $q(CO_2)$ were found from the 1st to the 30th day of the experiment, primarily as a result of the harmful effect of atrazine. The time interval of high $q(CO_2)$ values coincided with the time of significant changes in dehydrogenase, biomass carbon and intensity of respiration. Later (60 days after application), atrazine toxicity ceased, primarily due to its degradation, as well as to the restoration of the populations of living organisms which had adjusted to the new conditions and were able to utilize atrazine molecules as a source of available nutrients and energy for their physiological processes.

CONCLUSIONS

Atrazine was found to cause different effects on the biochemical activity in soil (*i.e.*, dehydrogenase, biomass carbon, respiration and metabolic coefficient). Its influence depended on the application load and duration of activity, and was either stimulating or inhibitory. The impact of atrazine on dehydrogenase activity was consistently negative for each herbicide concentration and depended on the application load. Decreased activity of dehydrogenase under all atrazine concentrations was observed until the 30th day after application. A negative effect was not detected at the end of the incubation period (60th day), when atrazine treated and untreated soils showed similar dehydrogenase values. On the basis of the microbial biomass carbon, soil respiration and metabolic coefficient, non-consistent positive or negative atrazine effects were observed and the effects persisted until the 60th day.

The results suggest difficulties in using biochemical parameters as indicators of the impact of atrazine on soil as different results were acquired depending on the biochemical parameter examined, the application load and post-treatment time. Of the examined parameters, dehydrogenase activity seemed to be the most useful indicator of impact of atrazine on soil environment.

In conclusion, it should be noted that the investigated loadings were either recommended or multiple doses and the observed changes were temporary in char-

acter and intensity, which suggests that there is no real risk from atrazine of causing a disruption of the existing balance of soil biochemical processes.

ИЗВОД

ДЕЛОВАЊЕ АТРАЗИНА НА НЕКА БИОХЕМИЈСКА СВОЈСТВА ЦРНИЦЕ

Љ. РАДИВОЈЕВИЋ, С. ГАШИЋ, Љ. ШАНТРИЋ и Р. СТАНКОВИЋ-КАЛЕЗИЋ

Институт за пестициде и заштиту животне средине, Банатска 31б, п. пр. 163, 11080 Београд

У раду је испитивано деловање пестицида (атразина) на биохемијску активност земљишта. Оглед је постављен у лабораторијским условима на земљишту типа глиновита иловача. Атразин је примењен у количинама од 8,0, 40,0 и 80,0 mg/kg земљишта. Праћена је активност ензима дехидрогеназе, биомасе угљеника, респирација (дисање) земљишта као и метаболитички коефицијент. Узорци за анализе узимани су 1, 7, 14, 21, 30 и 60 дана после примене атразина. Добијени резултати су показали да је деловање атразина на биохемијску активност земљишта зависило од примењене количине и дужине деловања, те је у зависности од тога, било стимулативно или инхибиторно. Међутим, утврђене промене су биле пролазног карактера, тако да може да се сматра да нема реалног ризика од нарушавања равнотеже биохемијских процеса у земљишту под утицајем овог једињења.

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