The effects of fluazinam and flusulfamide on soil ATP-microbial biomass

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Abstract

The effects of two fungicides, fluazinam, a pyridine fungicide and flusulfamide, a sulfoannilide fungicide, on soil microbial biomass as estimated through soil ATP content has been assessed. The fungicides were applied on soils under study at a concentration level 3000 mg/kg. The progress of soil microbial biomass changes were evaluated at 1st, 3rd, 7th, and 14th day after the pesticides implementation based on the fluorescence intensities of soil ATP emitted at a wavelength range 510 – 670 nm. Fast reduction of soil ATP content up to 60% (from 0.143 to 0.058 nmol/g soil) was detected after one day of flusulfamide application on soils. However, gradual recovery of soil ATP content initiated at day-3 after the application of such a fungicide. On the other hand, the application of fluazinam on soils demonstrated lack of capability of soil ATP content to recover until the end of the experiment period. These findings suggested that fluazinam might induce prolong deleterious effects to soil health and soil quality compared to flusulfamide and thus might draw concerns on environmental problems.

Keywords: xenobiotics, fluazinam, flusulfamide, soil ATP, soil health

Introduction

It has been well known that pesticides can significantly improve agricultural production by protecting plants from diseases. However, it is also renowned that some of the applied pesticides might induce deleterious effects to the environmental sustainability, particularly on soil health and soil quality.

Recently EPA has categorized two fungicides fluazinam (3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine) and flusulfamide (2′,4-dichloro-α,α,α-trifluoro-4′-nitro-m-toluenesulfonanilide) as “suggestive evidence of carcinogenicity, but not sufficient to access human carcinogenic potential” (PAN UK 2005, Neumiester & Reuter 2008). The two fungicides have been used extensively to counter attack the outbreak of Plasmodiophora brassicae or commonly known as clubroot disease. Such a fungal disease causes the roots of plants to swell asymmetrically resulting in the roots are unable to absorb water and nutrients appropriately.

Fluazinam (Figure 1, chemical structure A) is classified as a pyridine fungicide while flusulfamide (Figure 1, chemical structure B) is classified as a sulfoanilide fungicide. Both are xenobiotics with physico chemical properties as presented in Table 1. Although the purpose of fungicides application on agricultural sites is to alleviate the emergence of pests, however, several facts have demonstrated that non-target species are also become endangered. Often, the effects are so prominent as such creating other problems such as decreasing soil productivity and inducing environmental pollutions. Therefore, assessing to what extent the application of such fungicides might modify the sustainability of soil health and soil quality is very important.

There are several possible parameters, such as soil enzyme activities, soil respiration, soil microbial biomass, as well as soil microbial diversity that can be monitored to assess the changes of environmental quality. Among of them the determination of soil microbial biomass is frequently utilized for such a purpose. Soil microbial biomass serves as a dynamic pool for sustainability of nutrient cycling in soils and also as a measure of the living component in soils. For that reasons the parameter was selected to be used to evaluate the impacts of fluazinam and flusulfamide applications on soils.

Materials and Method

Soil microbial biomass is considered part of the organic matters in soils that constitute living microorganisms and occupy volume less than 5 µm3. In most soils, the proportion of soil microbial biomass ranges from 1 to 5% of soil organic matter. The latter might range from 1.5% (in loamy sandy soils) to 4.5% (in loamy soils).

Several methods have been utilized to estimate soil microbial biomass levels such as staining and counting the microbial cells, analyzing soil physiological parameters such as ATP, respiration, and heat output, and fumigating with chloroform and then analyzing the cytoplasm of the lysed cells (Abelho 2005, Anderson & Domsch 1978, Bölter 1994, Paul & Johnson 1977, Solaiman 2007). In this research the soil physiological
parameter assessment was employed to estimate soil microbial biomass through soil ATP level prediction.

In this research, both, fluazinam and flusulfamide were applied to soils at rate 3000 ppm. Soils for this experiment were taken from an experiment lot at HPTCAFT located in Hyogo Prefecture Japan. Before applying the two fungicides the soils were sieved on 2 mm screen and then followed by moisture adjustment at level 50% of water holding capacity. Next, 36 polycarbonate bottles of volume 250 mL that equipped with airtight lid were prepared and then 75% of each bottle capacity was filled with soils. Among of them, 12 bottles were served for triplicates controls of 1st, 3rd, 7th, and 4th-day of incubation and the rest bottles were served for triplicates of fluazinam and flusulfamide treatments at the aforementioned period of incubation. Prior additions of the two fungicides soil within the bottles were pre-incubated in dark condition for two weeks at temperature 25 °C to allow for the soil microbes to recover from stress and capable of adapting to the experiment conditions. This step was very important as sieving may affect soil microbial biomass determination (Ross et al 1985) as such may provide confounding effects to the treatment being applied and consequently the determination of soil biomass may give misleading interpretation.

At the end period of pre-incubation the bottles’ lid were opened and 12 bottles were poured with fluazinam and the other 12 bottles were poured with flusulfamide. Both fungicides were applied in the form of powder. After manual plowing to distribute fungicides evenly in soil matrix then further incubation at 1st, 3rd, 7th, and 4th-day of incubation and the rest 14-day of the incubation course the soil ATP level decreased significantly from 0.143 to 0.058 nmol/g soil. This is approximately 60% soil ATP reduction of that initial value at Day-0. The pattern of decreasing of soil ATP content continued up to Day-3 of the assessment after which soil microbial levels did not recover completely.

Similarly, the pattern of decreasing of soil microbial biomass was also recognized following the treatment of fluazinam. However, unlike flusulfamide, until the end of incubation period no turning point of recovering process of soil microbial biomass was observed. It might be that the fluazinam induce more deleterious effects on soil health and soil quality compare to flusulfamide.

**Results and Discussion**

The principal of measurement of soil ATP content is based on the following two steps of chemical reactions:

\[ \text{luciferin + ATP} \rightarrow \text{luciferil adenylate + PP} \quad (i) \]
\[ \text{luciferil adenylate + O}_2 \rightarrow \text{oxyluciferin + AMP} \quad (ii) \]

When the second reaction proceeds a fluorescence light would emanate from the reaction system. The wavelength of light given off is between 510 and 670 nm (i.e. pale yellow to reddish green color).

Table 2 demonstrates statistical results of soil ATP estimation and Figure 2 shows the pattern of soil ATP changes during the course of incubation. As can be seen in Figure 2 at Day-1 after flusulfamide addition the soil ATP level decreased significantly from 0.143 to 0.058 nmol/g soil. This is approximately 60% soil ATP reduction of that initial value at Day-0. The pattern of decreasing of soil ATP content continued up to Day-3 of the assessment after which soil microbial levels did not recover completely.

Interestingly, as demonstrated by Figure 2, soil controls that obtained no fungicides addition treatments showed fluctuating increment of soil ATP levels. All soils in this experiment have been
mechanically treated in order for evenly distributing fungicides powder throughout the soil matrix. This might be the cause of the dramatic increased of soil ATP level at Day-1 as such more than two fold of the level at Day-0. Even, at the end of the experiment the level of controls’ soil ATP levels did not turn back to the initial value. It has been known that soil plowing may induce environmental perturbation on soil ecosystem. As a result of environmental stress, in some soils, rate of soil basal respiration would increase. Such an increase demonstrated that the activities of soil microorganism enhanced as a respond to environmental changes.

Some studies that confirmed such a finding stated that the enhancement of soil microbial activities resulting in more respiration rates being performed by soil microbes in order to adapt with a new situation. Thus, more ATP will be synthesized in order to conduct faster rate of respiration. Nevertheless there were some studies that found decreasing of soil basal respiration was detected along with increasing the pollutant levels. Such an ambiguity respond might be overcome by study of substrate (usually glucose) induce respiration (SIR). The study of SIR on contaminated soils demonstrated that the digestion of substrates delayed for a certain period that depended on the level of contaminants in soils. The higher levels of contaminants induced prolong of lag phase of the releasing carbon dioxide compare to soils contaminated with lower levels of pollutants.

Table 1 Physical and chemical properties of fluazinam and flusulfamide

<table>
<thead>
<tr>
<th>Physical and Chemical Data</th>
<th>Fluazinam</th>
<th>Flusulfamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vapour pressure (Pa)</td>
<td>$2.3 \times 10^{-7}$ (25°C)</td>
<td>0.99 Pa (40°C)</td>
</tr>
<tr>
<td>Henry’s constant (atm m$^3$/mole)</td>
<td>$6.73 \times 10^{-7}$</td>
<td>-</td>
</tr>
<tr>
<td>Solubility in water (mg/L)</td>
<td>0.157</td>
<td>2.9</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.76</td>
<td>1.019</td>
</tr>
<tr>
<td>Octanol/water partition (log K_{ow})</td>
<td>2.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Adapted from Anon 2006 and Alternative Strategies and Regulatory Affairs Division-Pest Management Regulatory Agency 2003.

Table 2 Soil ATP levels following 1st, 3rd, 7th, and 4th-day after flusulfamide and fluazinam treatments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Flusulfamide</th>
<th>Fluazinam</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (nmol/g soil)</td>
<td>SD</td>
<td>ATP (nmol/g soil)</td>
<td>SD</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.122</td>
<td>0.006</td>
<td>0.143</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.316</td>
<td>0.032</td>
<td>0.058</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.206</td>
<td>0.036</td>
<td>0.038</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.185</td>
<td>0.040</td>
<td>0.044</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.234</td>
<td>0.068</td>
<td>0.060</td>
</tr>
</tbody>
</table>

Figure 2 Soil ATP contents following 1st, 3rd, 7th, and 4th-day of incubation after treated with 3000 ppm of fluazinam and flusulfamide.
Examining the log K\text{ow} of fluazinam and flusulfamide as presented in Table 1 indicates that both fungicides have a tendency to bioaccumulate in the organic rich environmental compartment (Connell 1990). Such values demonstrate that the two fungicides prefer to be present in a lipid phase than in a polar medium such as water. It is expected that at equilibrium the concentration of the two fungicides in a lipid (or the like) compartment might exist at levels more than 200 fold than that are present in water or soil solution. This means the cell of soil microbes that consisted of lipids may absorbed the fungicides and the chemicals might interfere with biochemical process within cells of soil microorganisms. Fluazinam has been known to interfere with oxidative phosphorylation, a mechanism of energy generation within living organisms. The xenobiotic acts as an uncoupling agent of electron-transport system within membrane cells. As the result the protonmotive force will be destroyed and ATPase loss capability to synthesize ATP from ADP (Pelczar, Jr. \textit{et al} 1993). Thus, such an interfere is suspected to be the cause of decreasing soil ATP levels and failure to recover. However, it is not clear whether similar actions occur for flusulfamide.

Similarly, solubility data of fluazinam and flusulfamide demonstrate that the two fungicides hard to dissolve in water (mostly, organic chemists define organic molecules are water soluble when at least 3 g of the substance dissolve in 100 mL water, Solomon 1990), such that both chemicals might accumulate at the sites of application.

Although the ecotoxicological aspects of fluazinam have been studied intensively (e.g. PAN website), however, such information for flusulfamide is still lacking. Therefore further assessment on flusulfamide is necessary to be taken into account in order to elucidate the fate of the fungicide in the environment as well as in biota compartment.

Conclusions

Form this study it is concluded that fluazinam and flusulfamide might have a potential to alter soil health and soil quality. Fluazinam indicated to impose more deleterious effects to soil ecosystem than flusulfamide. The former xenobiotic demonstrated to reduce soil ATP levels and may impose adverse effects to soil microbial function.

Acknowledgements

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References


