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Laboratory Study on the Effects of Artificial Acid Rain on *Armadillidium vulgare* (Armadillidiidae, Isopoda)

Kajian Laboratorium Tentang Pengaruh Hujan Asam Buatan terhadap *Armadillidium vulgare* (Armadillidiidae, Isopoda)

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Abstrak

Kajian laboratorium ini dilakukan untuk mengetahui mekanisme pengaruh negatif hujan asam terhadap makrofauna tanah. *Armadillidium vulgare* yang merupakan salah satu makrofauna tanah pemakan seresah (*saprofag*) penting di Tokyo yang digunakan dalam penelitian ini. Pengaruh makanan, pengaruh langsung dan pengaruh medium yang direndam dengan cairan asam digunakan untuk mengetahui mekanisme ini. (1). Pengaruh makanan dilakukan dengan cara merendam seresah daun *aoki* (*Aucuba japonica*) dalam air atau air yang diberi cairan H_2SO_4 dengan pH 6,4, dan 2. Seresah ini diberikan pada *A. vulgare* sebagai makanan (2) Efek langsung dilakukan dengan cara meneteskan 1 ml air atau air yang diberi cairan H_2SO_4 langsung ke tubuh hewan setiap dua minggu. (3) Pengaruh medium dilakukan dengan merendam medium dalam air maupun air yang diberi cairan H_2SO_4. Pertumbuhan dan persen hidup *A. vulgare* dicatat. Pertumbuhan dan persen hidup *A. vulgare* yang diberi makan seresah yang rendam dalam larutan asam sangat rendah. Penetesan cairan asam pada tubuh *A. vulgare* secara langsung tidak berpengaruh negatif terhadap pertumbuhan dan persen hidup *A. vulgare*. Pertumbuhan *A. vulgare* tidak dipengaruhi oleh medium yang direndam dalam cairan asam, tetapi persen hidupnya sedikit berkurang. Pengaruh cairan asam secara tidak langsung melalui makanan lebih penting daripada pengaruh langsung. Hal ini disebabkan tubuh *A. vulgare* dilindungi kutikula yang tebal. Untuk taksa yang memiliki tubuh yang lunak pengaruh langsung mungkin juga penting.

Kata kunci : Hujan asam, *Armadillidium vulgare*, pertumbuhan, dan persen hidup

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Introduction

Acid rain is becoming one of the most important environmental issues. Acid rain is defined as rain or snow having a pH value below of 5.6. Human activity, especially fuel burning can emit $SO_2$and NO_2 to the atmosphere. Hydroxyl radicals, in turn, convert nitrogen dioxide into nitric acid and initiate sulfur dioxide into sulfuric acid (Begon *et al*., 1996; Likens *et al*., 1979; and Mohnen, 1988). In Japan, the increase of acidity in rain has become a matter of concern in recent years and the average pH value of rain is between 4.4-5.9.

Soil can be affected by acid rain. Nutrients such as calcium, magnesium, and potassium may be leached by acid rain. Low soil pH and a high concentration of aluminum can reduce populations of bacteria that breakdown and release nutrients from organic matter (Mohnen, 1988). Garden and Davis (1988) show this microbial activity on leaf litter.

Soil fauna is an important factor in the soil ecosystem; it plays a crucial role in humification and mineralization of organic matters, soil mixing and turnover. According to Luxton (1982), soil invertebrates function by fragmenting dead organic matter and increasing the surface area available for microorganisms.

Many studies on the relationship between acid rain and soil microfauna have been done in Europe (Hagvar, 1984; Hagvar and Amundsen, 1981; Hagvar and Kjondal, 1981). However there are only a limited number of studies dealing with the effects of acidic water on the soil macrofauna (Gunnarson, 1987; Zimmer and Top, 1997; Furuta e*t al*., 1997). Furuta *et al*., (1997) showed that in a woodland of Tokyo taxa Isopoda was the most adversely affected by artificial acid rain.

A laboratory study is needed to understand the mechanism of this adverse effect of acidic water on soil macrofauna especially taxa Isopoda. *Armadillidium vulgare* (Armadillidiidae, Isopoda) is one of the most important saprophagous soil macrofauna in cities in Japan. This study was aimed at understanding the direct and indirect ways that acid rain affects *A. Vulgare*.

Materials and methods

The study was done at the Laboratory of Forest Zoology, The University of Tokyo, Japan. Four experiments namely 1. Food choice experiment; 2.The effects of acidic water treated food to *A. vulgare;* 3. Direct effects of acidic water; 4. Effects of acidic water treated media were done in the study.

Food choice experiment

Dry leaf discs of *Aucuba japonica* (*aoki*) 1.3 cm in diameter were soaked in tap water (pH 7.5) or three levels of acidity of sulfuric solution (pH 6, 4, and 2) for one week and dried at temperature 60ºC for two days. The leaf disc, one piece for each treatment, was put in a plastic pot, 6 cm in height and 13 cm in diameter, at more or less equal distance. *A. vulgare*, body weight 39.9 mg ± 7.2 mg, was put into the plastic pot individually after being starved for one day. sixteen replications were made in this study. The plastic pots were placed in an incubator at 22±1^{0}C for three days. The leaf remnant was re-dried and the amount of leaves ingested was determined.

The effects of acidic water treated food to *A. vulgare*

Dry aoki leaves were soaked in tap water (pH 7.5) or acidic water (pH 6, 4, and 2) for one week and then air dried. Young individuals, age of approximately 2 months and body weight between 4.2 mg - 8.7 mg, were used and they were cultured in organic matter in room temperature before being used. One individual was placed in a plastic pot, of which base was covered by moist filter paper. To maintain the humidity, moist folded-tissue paper covered by aluminium foil was placed over the tissue paper. The litter was put into the pot as food. Seventeen replications were made for each treatment. The pot was placed in an incubator at temperature 22ºC in continuous darkness. The growth and the mortality was followed.

Direct effects of acidic water

A. vulgare was collected from the field. The animals were reared individually in a plastic pot filled with decomposed tree bark of oak to the depth±1 cm. one ml of acidic water (pH 6, 4, and 2) and tap water was dropped directly to the body of the animal in every two weeks. Moist folded tissue paper was placed inside the plastic pot to maintain the humidity and dry litter of Aoki was added as food. Twenty replications were made in the study. The plastic pots were put in incubator at temperature 22 ^{0}C for 5 months. The mortality and the growth percentage were determined.

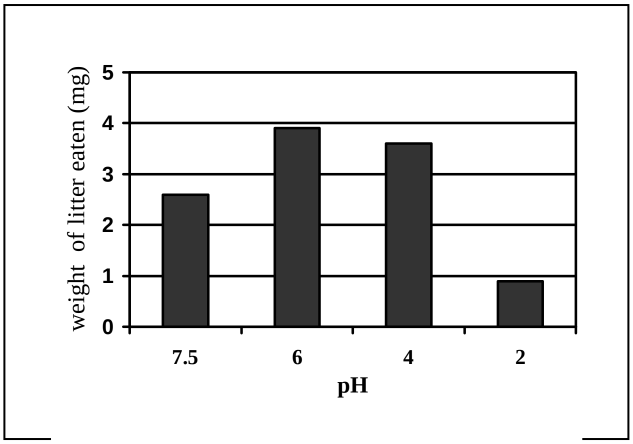
Effects of acidic water treated media

Decomposed organic matter (oak bark) was soaked in tap water (pH 7.5) or acidic water (pH 6, 4, 2.7 and 2.2) for one week. The media was then dried in the oven 60^{0}C for four days. Moist folded tissue paper was placed inside a plastic pot to maintain the humidity. One individual of *A. vulgare* 9.4±0.6 mg was placed in each plastic pot and aoki litter was added as food. The pots were placed in an incubator at temperature 22^{0}C. The mortality and the growth were determined. Twenty replications were made in the study.

Results

Food choice experiment.

Results of the food preference experiment of *A. vulgare* are shown in Figure 1. The leaves treated with pH 6 solution were the most preferred, followed by pH 4 and tap water (pH 7.5). The animals ingested very small quantity of the leaves treated with pH 2 solution and it was significantly different with pH 6 treatment ($p<0.05$).



Figu ... itter discs treated with tap water (pH 7.5) or acidic water (pH 6, 4, and 2) expressed by dry weight of litter disc consumed

Effects of acidic water treated food to juvenile of *A. vulgare*.

The effects of acidic water treated food to the growth and survival are shown in Figure 2a and 2b. The survival rate in tap water treatment was the highest, followed by pH 4, 6, and 2 (Fig. 2a). The individuals offered with tap water treated food showed a higher growth than those offered acidic water treated food but only pH 2 was significantly different from the first week to the fifth week ($p<0.01$). The growth of the animals in pH 4 was significantly lower than in tap water in the fourth week ($p<0,05$) but not in the fifth week. After 5 weeks the fauna grew 50.2 %, 43.8%, 35.9% and -2.3% from the initial weight in tap water and acidic solution pH 6, 4, and 2 respectively (Fig. 2b).

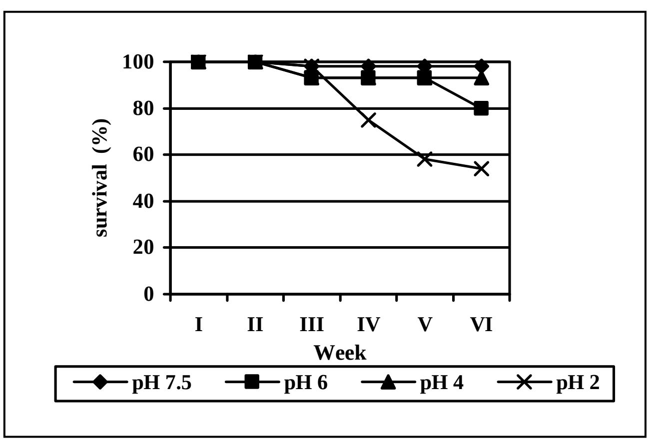


Figure 2a. Survivorship of *A. vulgare* (%) offered litter treated with tap water (pH 7.5) or acidic water (pH 6, 4, and 2)

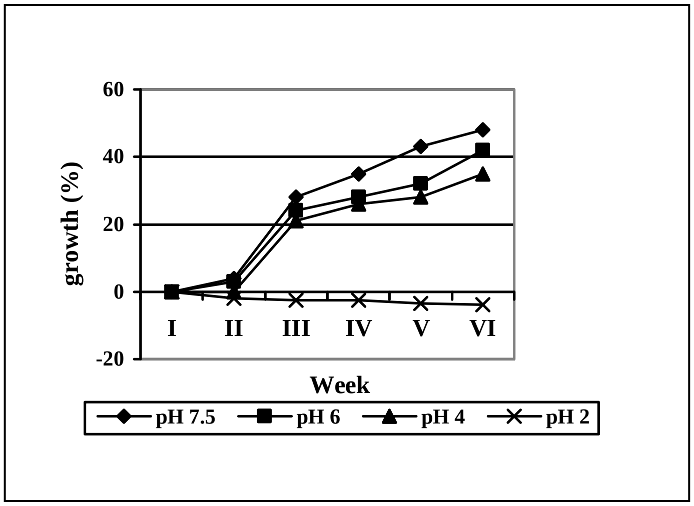


Figure 2b. Growth percentage of *A. vulgare* (%) offered litter treated with tap water (pH 7.5) or acidic water (pH 6, 4, and 2).

Direct effects of acidic water

At the end of the treatment the survival rate was similar between treatments.The survival rate in all treatments was higer than 90 % (Fig. 3a). The growth rate in the most acidic concentration showed no negative effects of acidic water, and surprisingly the growth rate was slightly higher than tap water (3b). But no significant difference was observed between treatment. After more than two months, the growth was more than 200 percent from their initial weight. Direct application of acidic water on the body of *A. vulgare* seem to have no significant effect.

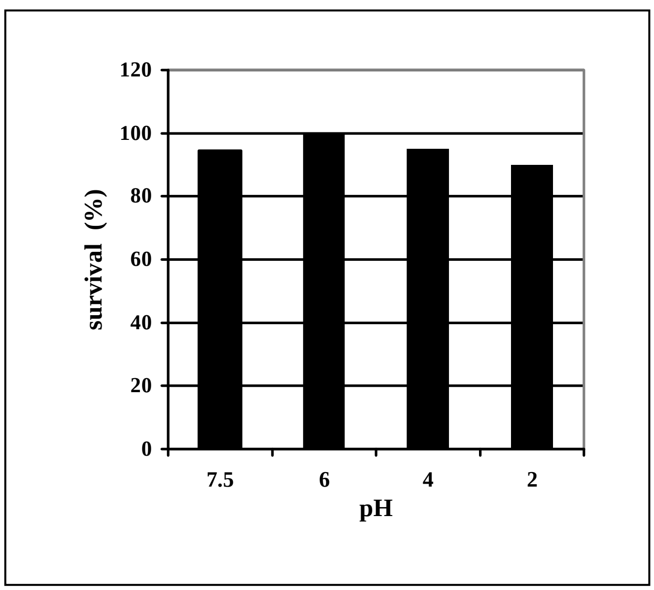


Figure 3a. Survivorship of *A. vulgare* (%) treated by dropping 1 ml tap water (pH 7.5) or acidic water (pH 6, 4, and 2) directly onto the body of the animal

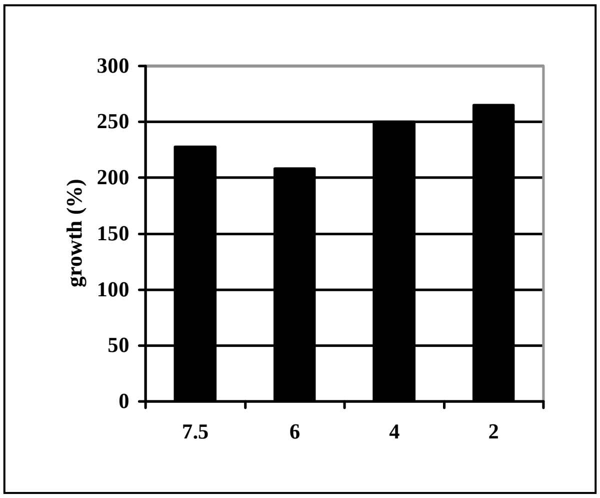


Figure 3b. Growth percentage of *A. vulgare* (%) treated by dropping 1 ml tap water (pH 7.5) or acidic water (pH 6, 4, and 2) directly onto the body of the animal

Effects of acidic-water-treated media

Acidic-water-treated media seemed to affect the survival rate of *A. vulgare* (Fig. 4a). At the end of the experiment, the survival rate was very low in the most acidic water treatment (pH 2.2). Only 35% of the individuals survived in this treatment. Tap water treatment and less acidic water treatments had high survivorship in which more than 80% of the animals survived the experiment. The growth rate of the animals was also the lowest in the media treated with the highest concentration of acidic water different between sites (Fig 4b). Statistical analysis, however, showed no significant difference between treatments.

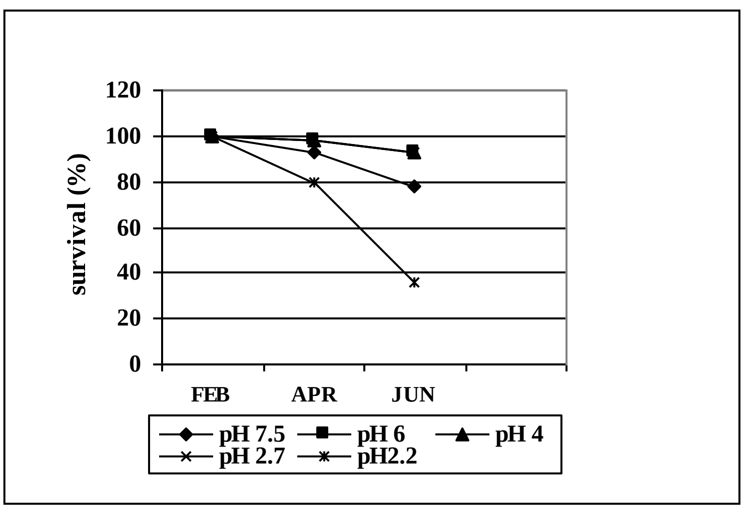


Figure 4a. Survivorship of *A. vulgare* (%) cultured in organic matter treated with tap water (pH 7.5) or acidic water (pH 6, 4, 2.7 and 2.2)

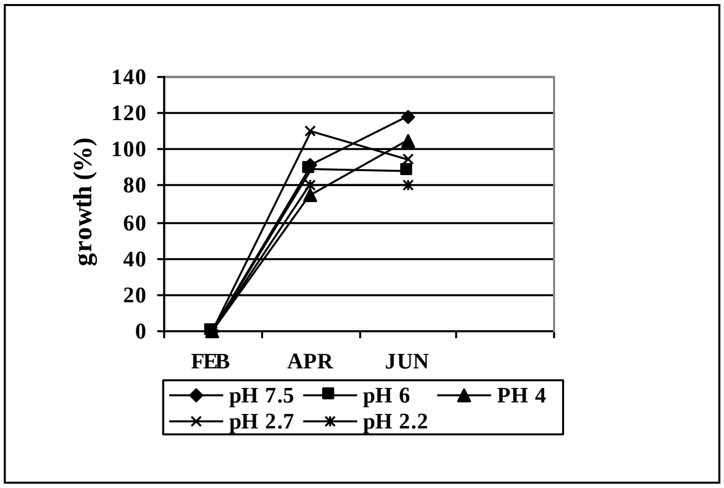


Figure 4b. Growth percentage of *A. vulgare* (%) cultured in organic matter treated with tap water (pH 7.5) or acidic water (pH 6, 4, 2.7 and 2.2)

Discussion

In the laboratory study *A. vulgare* preferred food treated with tap water or moderate acidic water (pH 6 and 4) to that treated with strong acidic water (pH 2). The strong acidic water may affect the physical and chemical conditions of the leaves and makes the leaves unpalatable. The animals may react directly to the acidity or to ion SO_2. Zimmer et *al*. (1996) pointed out that *Porcellio scaber* (Oniscidae, Isopoda) could perceive food by olfaction. The fauna does not react to the litter itself, but to the odor of some metabolites of the organisms colonizing the leaves.

Garden and Davis (1989) showed that microbial activity on leaf litter exposed to pH 3 and 4 was lower than that treated with pH 5.4. Gunnarson (1987) showed that *Oniscus acellus* (Isopoda), fed on the leaves of maple, preferred the area colonized by microorganism. In this study microorganism colonization may be low in the litter treated with strong acidic water. Therefore, *A. vulgare* avoided strong acid treated food.

The growth and survival rate of young *A. vulgare* decreased when they were offered acidic water treated food. Young individuals were sensitive to the environment or food quality. Therefore, young *A. vulgare* were easily affected by the acidic water treatment. There are at least two possible explanations for the fact that the juvenile animals had a decreased growth and survival when they were offered acidic water treated leaves. Firstly, the animals do not eat enough food and starve, because they avoid ingesting strong acidic water treated food. Secondly, the quality of the food decreases. The leaves become physically tougher and acidic water may leach calcium, magnesium, sodium, and potassium and also negatively affect nitrogen content of the leaves. Acid treatment is associated with accelerated leaching of Ca^{2+}, Mg^{2+}, K^{+}, Mn^{2+} (Brown, 1985). According to White (1978) for many if not most animals, the single most important factor limiting their abundance is the relative shortage of nitrogenous food for their young. Foliar nitrogen and phosphour decrease with decreasing of pH (Garden and Davis, 1988). The result seems to agree with the study by Zimmer and Top (1997) that showed that acidification affected the mortality of the female *Porcellio scaber* (Isopoda) and longevity of juvenile, correlated with microorganism in the litter.

Treatment by dropping the acidic solution directly to the body of the animals did not show any negative effects to *A. vulgare.* The body of *A. vulgare* was protected by thick cuticle that may function protecting the fauna. Chapman (1998), noted that the cuticle can protect insect from abiotic environment. The solution may also dry up quickly so the effects may not be significant. The growth of *A. vulgare* was not affected when reared in the media treated with acidic water, but the survival rate was affected by the most acidic solution. The fauna may still grow in the acidic media when offered with untreated food. Direct contact to acidic media for longer period may be harmful to *A. vulgare*.

Recommendation

Acidic water can decrease the quality of litter as food of *A. vulgare*. To conserve *A. vulgare*, an important litter decomposer in urban woodland, it is recommended to improve the quality of the litter and mitigate the negative effects of acid rain. Liming should be done in the floor of woodland to increase the pH of the soil and it may improve the quality of the litter.

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