The Toxic Action of Certain Chemicals on Aquatic Oligochaetes.

By

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Introduction.

While experimenting on the toxic action of *Leucothoe grayana* MAX. *(hanahiri-no-ki)* on certain aquatic oligochaetes*, the writer found that potassium hydroxide has a considerable toxicity, that the extract of *hanahiri-no-ki* which was prepared using a solvent containing potassium hydroxide at the rate of 0.04% was slightly acid in reaction and also that the extract prepared in this manner did not differ in toxicity from the extract to which no potassium hydroxide was added[1].

These facts raised the following question: Does the solution of potassium hydroxide owe its toxicity to the hydroxide-ion or to the potassium-ion? How high is the toxicity of the solution of potassium hydroxide to the aquatic oligochaetes? To answer these questions, the writer conducted some experiments in 1930—1931 and found that the solution of potassium hydroxide has a strong toxicity to the aquatic oligochaetes.

If the potassium-ion is responsible for the toxicity of potassium hydroxide solution, then potassium nitrate, which contains potassium as one of its constituents, must also be toxic to the aquatic oligochaetes. This substance can be used as a fertilizer. If it is also effective as a vermicide against the aquatic oligochaetes, it will be of two-fold benefit to the farmer; namely, first as a fertilizer and secondly as a vermicide. Accordingly, the writer also decided to study the toxic action of potassium nitrate.

If, on the other hand, the toxicity of potassium hydroxide is due to the presence of hydroxide-ion in the solution, calcium hydroxide solution must also be toxic to the aquatic oligochaetes. That the solution of quicklime is strongly toxic to the aquatic oligochaetes has already been shown by INUKAI**. According to the result of his experiments, a certain Limnodrilus species died in 40 minutes when immersed in a 0.01% solution of quicklime[2].

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* The aquatic oligochaetes which were used for the experiments were *Limnodrilus gotoi* HATAI and an unidentified species belonging to the genus Branchiura. In Japan, these oligochaetes are called "*yuri-mimizu*". They sometimes do considerable damage to the rice-seedlings in the seed-bed.

** Dr. INUKAI of the Hokkaido Imperial University kindly called the attention of the writer to his experiment which had been carried out several years ago.

In consideration of these facts the writer carried out some experiments on the toxic action of potassium hydroxide, potassium nitrate and calcium hydroxide. The results obtained are reported below.

Materials and Method Used.

Experiments were conducted both in the laboratory and in the field. The laboratory experiments were carried on by the use of a constant temperature water tank, the temperature of the water being kept at approximately 25—26.5°C. The field experiment was conducted in a rectangular wooden frame of one-fourth *tsubo** in area and 33 cm. in height. The lower two-thirds of the frame was buried in the soil of the rice-field.

The test animals used for the experiments in the laboratory were an unidentified species of aquatic oligochaetes belonging to the genus Branchiura, while those used for the field experiments consisted of the Branchiura species and a small number of a species of Limnodrilus.

In the field experiments, the number of worms which were alive or dead was not counted to determine the percentage of dead worms. A rough estimation was made simply by inspecting the dead worms on the surface soil inside of the frame. Consequently, only a rough approximation of the percentage of dead worms was obtained. However, this was considered sufficient for the purpose since the writer's chief object was to rate the relative toxicity of the materials necessary to kill 100% of the test animals.

Laboratory Experiments.

Survival time of the Branchiura in distilled water.

Several worms were placed in distilled water and kept in the constant temperature water tank for more than a week, but no worms died although they became much thinner than they were at first. At the end of a week, all were still active and it seemed that they would continue to remain active for a much longer time. It may be assumed, therefore, that distilled water has no injurious effect upon the Branchiura worms during the course of experiments concluded within a period of two or three days.

Toxicity of potassium nitrate.

Solutions of potassium nitrate in various concentrations were used to study its toxic action on the Branchiura worms. The results of the experiments are shown in Table I.

* One *tsubo* ≑ 3.3 square meters.

Table I.

Toxicity of Potassium Nitrate to Branchiura.

Concentration (%)	Duration of Immersion (Minutes)	Average Percentage of Dead Individuals	Remarks
0.05	480	35	
0.1	〃	100	Majority ceased movement in 2 hours.
0.2	300	55	Movement stopped in 50 minutes.
0.3	〃	100	
〃	180	50	
0.5	120	93	
〃	60	45	
1.0	〃	65	Majority ceased to move in 10 minutes.

According to the results shown in Table I, the Branchiura worms ceased to move in a rather short time when they were immersed in the solution of potassium nitrate. However, the toxicity of the solution can scarcely be considered very high since nearly 35% survived immersion for 60 minutes in a 1% solution of potassium nitrate.

Toxicity of potassium hydroxide.

The results of the experiments conducted with potassium hydroxide are shown in Table II.

Table II.

Toxicity of Potassium Hydroxide to Branchiura.

Concentration (%)	Duration of Immersion (Minutes)	Time elapsed before Movements stopped (Minutes)	Per Cent. Dead Worms	Remarks
0.001	420	More than 420 minutes.	0	None ceased to move within 420 minutes.
0.0016	120	More than 120 minutes.	0	None ceased to move.
0.0025	120	100	20	
0.005	120	—	75	
〃	60	24	80	
0.01	60	4	85	
〃	120	—	100	
0.02	60	2	100	
0.05	60	Almost instantly.	100	
0.1	60	〃	100	

According to the results shown in Table II, potassium hydroxide has a very high toxicity to the Branchiura worms. In a very weak solution containing potassium hydroxide at the rate of 0.02%, the test animals ceased to move in 2 minutes. Immersion of 2 hours in a 0.01% solution killed 100%.

Toxicity of calcium hydroxide.

Quicklime was used in preparing the solution of calcium hydroxide. For the sake of convenience, the concentration of the mixture was expressed as the percentage of quicklime which was contained in the mixture. Accordingly, when the percentage of calcium hydroxide is mentioned in later paragraphs, it refers to the percentage of quicklime in the sample mixture.

The results of the experiments conducted with calcium hydroxide are shown in Table III.

Table III.

Toxicity of Calcium Hydroxide to Branchiura.

Concentration (%)	Duration of Immersion (Minutes)	Time elapsed before Movements stopped (Minutes)	Per Cent. Dead Worms	Remarks
0.001	420	More than 420 minutes.	0	None ceased to move.
0.0016	120	$120+\alpha$	45	Some were still active 120 minutes after immersion.
0.0025	120	40	60	
0.005	60	—	100	
0.01	〃	9	100	
0.02	〃	3	100	
0.05	〃	3	100	
0.1	〃	Almost instantly.	100	

The results in Table III show that calcium hydroxide is highly toxic to the Branchiura worms. A 0.2% solution arrested the movements of the worms in only 3 minutes and immersion for 60 minutes in the solution of this concentration killed 100%. Still more striking was the fact that 100% were killed by immersion for 60 minutes in a 0.005% solution.

Comparative toxicity of potassium nitrate, potassium hydroxide and calcium hydroxide.

According to the results of experiments described above, the toxicity of potassium nitrate is considerably lower than that of the other two substances. For instance, a 0.05% solution of potassium nitrate killed only 35% when the Branchiura worms were immersed in it for 8 hours, while the solution of either potassium hydroxide or calcium hydroxide at the same concentration killed 100% by an immersion of only one hour.

Immersion for one hour in a 1% solution of potassium nitrate killed 65%, while in the case of the other two substances an immersion of the same duration in a 0.1% solution killed 100%. The difference is thus quite conspicuous.

Potassium hydroxide and calcium hydroxide will next be compared in regard to their toxicity to the Branchiura worms. In the case of potassium hydroxide, immersion for 2 hours in a 0.0016% solution did not kill any test animal, while similar treatment with the solution of calcium hydroxide at the same concentration killed 45% of the test animals. A 0.005% solution of potassium hydroxide killed 80% by immersion for 60 minutes, while the solution of calcium hydroxide at the same concentration killed 100% by an immersion of the same duration.

Next, we will consider the time necessary to arrest the movements of test animals. When the test animals were immersed in a 0.1% solution of either potassium hydroxide or calcium hydroxide, they ceased to move in a short time in either case. A closer observation revealed that the time which elapsed before the cessation of movements was slightly shorter in the solution of potassium hydroxide than in that of calcium hydroxide. In other words, the toxic action of potassium hydroxide was slightly stronger than that of calcium hydroxide. However, when the concentration of the solution became 0.005% or lower, the time required to arrest the movements became shorter in calcium hydroxide than in potassium hydroxide. For instance, at a concentration of 0.02%, the test animals ceased to move in 2 minutes in the case of potassium hydroxide while in the case of calcium hydroxide the time which elapsed before the cessation of movements was 3 minutes. When a concentration of 0.0025% was reached, 100 minutes were necessary to arrest the movements in the case of potassium hydroxide, while in the case of calcium hydroxide only 40 minutes were required. At a concentration of 0.0016%, the solution of potassium hydroxide could not arrest the movements of the test animals by immersion for 2 hours, while in the case of calcium hydroxide some of the test animals ceased to move when they were immersed in the solution for the same period of time.

The relative toxicity of the two substances is graphically compared in the following two figures. (See Fig. 1 and Fig. 2.)

Fig. 1.

Percentage of worms killed by immersion for two hours.

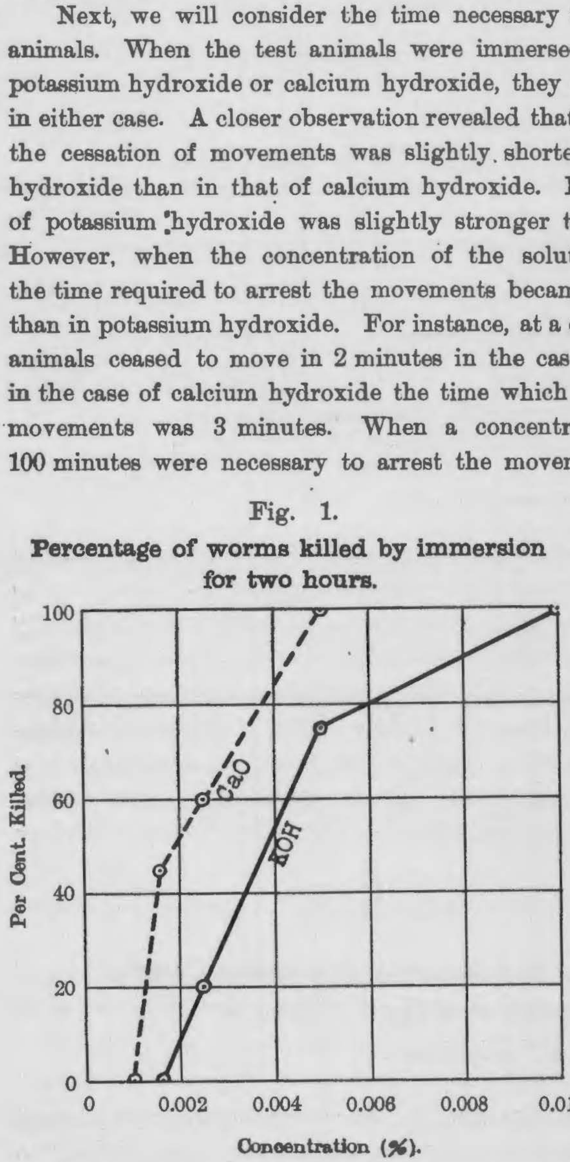

Fig. 2.

Time elapsed before the movements of worms ceased.

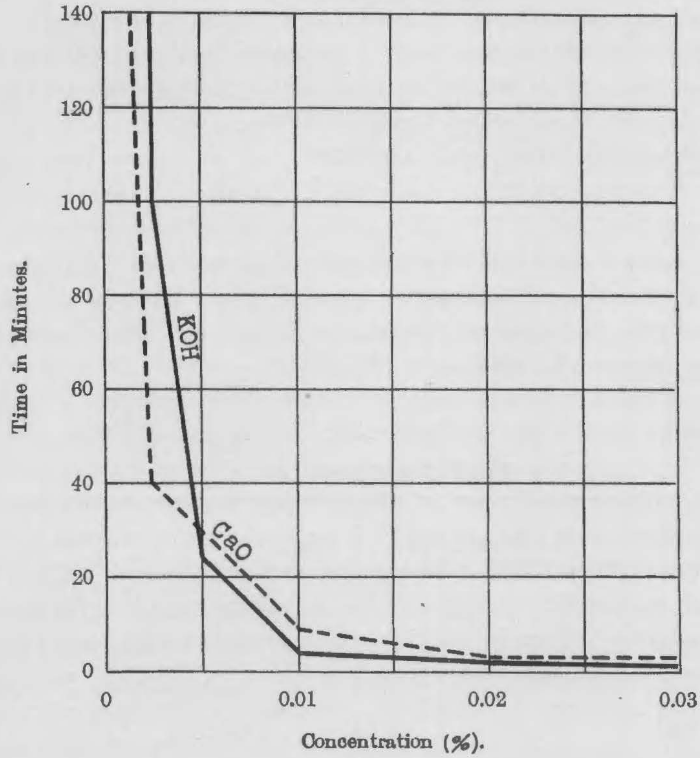

Figure 1 shows clearly that at a concentration of 0.005% or lower the toxicity of calcium hydroxide is higher than that of potassium hydroxide. According to Figure 2, the time required to arrest the movements of test animals is slightly shorter in potassium hydroxide solution than in calcium hydroxide solution when the concentration is 0.005% or higher. In other words, above a certain concentration the toxicity of potassium hydroxide is slightly higher than that of calcium hydroxide. However, it is evident from Figure 2 that when the concentration is 0.0025% or lower the time required to arrest the movements is slightly shorter in calcium hydroxide than in potassium hydroxide. This is just the same tendency as is shown in Figure 1.

Field Experiments on the Toxicity of Caustic Potash, Nitrate of Potash and Hydrated Lime to *Yuri-mimizu*.

The results of the experiments described in the previous paragraph showed that potassium hydroxide and calcium hydroxide had a conspicuous toxicity to the Branchiura worms. These results seemed to indicate the possibility of using these substances as vermicides against certain aquatic oligochaetes known as "*yuri-mimizu*". Accordingly, some experiments were carried out in the rice-field using potassium hydroxide, calcium hydroxide and potassium nitrate.

An unidentified species of the genus Branchiura constituted the majority of the test animals, but a small number of Limnodrilus species were mingled with them.

The experiments are briefly described below.

Experiment I.

A square wooden frame of 1/4 *tsubo* in area was used for each plot. The chemicals to be tested were dissolved or mixed in about 10 litres of tap water and poured into the frames upon the surface of the soil. The results of the experiment are presented in Table IV.

Table IV.

Field Experiment I.

Plots	Chemicals used	Concentration (%)	Amount of Chemicals used per *Tan** (*Kan***)	Results
I	KOH	0.005	—	Some 30—40% died.
II	〃	0.01	—	Nearly the same as in *Plot I.*
III	CaO	0.05	1.6	All died.
IV	〃	0.10	3.2	All died.
V	KNO_3	0.30	9.6	Barely 20 or 30% died.
Check				None died.

* One *tan* ≒ 992 square meters.

** One *kan* ≒ 3.8 kilograms.

The results noted from observations during the experiment were as follows: In *Plots I* and *II*, the worms entered the soil shortly after they were introduced into the frame and began the peculiar wavering motion with their tails which were kept in the water above the soil. No sign of excitement or of agony could be found. In *Plots III* and *IV*, the worms immediately ceased to move, began to bleed through the skin and died in a short time. In *Plot V*, the worms at first showed a sign of agony for a while, but the majority entered the soil and began the peculiar wavering motion with their tails.

The results noted at the last observation were as shown in Table IV. According to the results, in this experiment the toxicity of the solution of potassium hydroxide was not so conspicuously high as was observed in the laboratory experiment. In a laboratory experiment conducted under a constant temperature, a 0.01% solution of potassium hydroxide killed 100% by immersion for 2 hours, while in the field experiment we could kill only a small percentage of worms as it is apparent from Table IV.

Experiment II.

In this experiment, the water in the frame was drawn out nearly completely and the chemicals were mixed in the upper 5 or 6 centimeters of the soil in the frame. Water was poured into the frame until it reached about $1\frac{1}{2}$ centimeters above the soil surface and several hundred test animals were placed in the frame. The results of the experiment are shown in Table V.

Table V.

Field Experiment II.

Plots	Chemicals used	Amount of Chemicals used per *Tan* (*Kan*)	Results
I	KNO_3	16	Approximately 30—40% killed.
II	〃	20	Nearly the same as in *Plot I.*
Check			Apparently none died.
IV	CaO	8	Barely 20—30% killed.
V	〃	16	Nearly the same as in *Plot IV.*
VI	〃	20	Approximately 50% killed.

Contrary to the writer's expectation, the percentage of kill obtained with calcium hydroxide was rather low in the present experiment. The toxicity of calcium hydroxide seemed to be conspicuously lower in this experiment than in the previous one. The cause of the rather poor result obtained will be considered in a later paragraph.

Experiment III.

The method used in this experiment was quite the same as was used in the previous experiment. The results are shown in Table VI.

(See Table VI on next page.)

Observations following the introduction of the test animals showed that in *Plots I* and *II* the worms entered the soil in a short time and began the peculiar wavering motion in the water, while in *Plots V* and *VI* the worms wriggled about apparently in excitement and agony and all died about 20 minutes later.

The records in Table VI show the results of the last observation which was made the next morning. These results indicate that "*yuri-mimizu*" could be killed in a short time by using 40 *kans* of calcium hydroxide per *tan*. Potassium nitrate had a much lower toxicity than calcium hydroxide and could kill only a small percentage of worms even when the same amount (40 *kans*) was used.

Table VI.

Field Experiment III.

Plots	Chemicals used	Amount of Chemicals used per *Tan* (*Kan*)	Results
I	KNO_3	30	Approximately 30—40% killed.
II	〃	40	Nearly the same as in *Plot I.*
Check			Apparently none died.
IV	CaO	20	Approximately 60—70% killed.
V	〃	40	All killed.
VI	〃	60	All killed.

Summary and Conclusions.

Potassium hydroxide and calcium hydroxide have a high toxicity to certain aquatic oligochaetes known as "*yuri-mimizu*" in Japan. There is a difference in toxicity between the solutions of these two substances at a certain concentration, but the difference is rather small.

The Branchiura worms can be killed by immersing them in a 0.005% solution of calcium hydroxide for one hour. It is evident from this fact that the toxicity of calcium hydroxide is very high. Inukai reported that no "*ito-mimizu*" (a species of Limnodrilus) were killed by an immersion of 24 hours in a 0.005% solution of quicklime.

When the writer's result is compared with that obtained by Inukai it is evident that the Limnodrilus species are considerably more resistant to the toxic action of calcium hydroxide than are the Branchiura species.

The toxic action of calcium hydroxide to the aquatic oligochaetes, though very strong under laboratory conditions, is not so strong when the chemical is mixed with the soil in the rice-field. This reduction in toxicity seems to be accounted for chiefly by the decrease in the amount of free calcium hydroxide in the soil owing to the chemical and physical combination of calcium hydroxide with certain ingredients of the soil in the rice-field.

According to the writer's experiments, about 40 *kans* of quicklime per *tan* are necessary to completely exterminate "*yuri-mimizu*" in the rice-field, provided that the quicklime is mixed with the upper 6 centimeters of the soil.

Potassium hydroxide also has a high toxicity to "*yuri-mimizu*". The amount of this chemical necessary to kill all the aquatic oligochaetes in the rice-field was not determined. In view of its strong alkalinity, potassium hydroxide is considered to be unsuitable for use in the rice-field.

Potassium nitrate is markedly lower in toxicity than potassium hydroxide though these two chemicals contain potash as one of their constituents. This fact indicates that the strong toxic action of the solution of potassium hydroxide is not solely due to the presence of potassium-ion.

The solution of calcium hydroxide is not so strongly alkaline as that of potassium hydroxide. In spite of this fact, calcium hydroxide is highly toxic to the aquatic oligochaetes as has been stated in a previous paragraph. It is interesting in this connection to learn that washing soda (sodium carbonate) is only slightly toxic to the Limnodrilus species although the solution of this substance is also alkaline in reaction. It is not known what property is responsible for this marked difference in toxicity in these two substances, calcium hydroxide and sodium carbonate.

Literature Cited.

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