原　　報

Takahiko Sato : The Toxicity of Metallic Ions on the Activated Sludge and the Detoxication Effect of EDTA

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Summary

Using the Warburg manometer, the author investigated the effect of metallic ions on oxygen uptake of activated sludge and calculated the values of TC_{50} (toxic concentration 50, the concentration of toxic substance required to obtain 50% inhibition of the oxygen uptake of unintoxicated sludge). The obtained results were as follows.

1) The toxicity was decreased with the following sequence in the unit of ppm.

Cu^{2+}, Ag^{+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cr^{6+} and Pb^{2+}.

2) The detoxication effect by EDTA was observed with Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Hg^{2+}. But for Ag^{+} and Co^{2+}, it was small and for Cr^{3+} and Cr^{6+}, the effect was negligible.

Recently the pollution of public water of Japan considerably worsened and the treatment of industrial wastes become very important problem. The industrial wastes contain many kinds of wastes and the treatment suitable for each waste must be necessary. The toxic wastes, such as metallic or cyanide wastes, are the most difficult to treat.

There are many literatures concerning to the toxic effect of metallic ions on the sewage treatments. Rudolfs. *et al.* (1) reviewed comprehensively the effects of several toxic materials and industrial wastes on anaerobic and aerobic treatments, on the microflora and microfauna of streams, and on the BOD tests. Kawamura (2) also reviewed the effects of heavy metal ions on sewage treatments.

Heukelekian and Gellman (3), using Warburg manometers, investigated the effects of copper, nickel, zinc, cadmium, trivalent chromium, hexavalent chromium and cobalt on the oxidations of domestic sewage and activated sludge-sewage mixture.

The staffs of R. A. Taft Sanitary Engineering Center (4) investigated precisely the effects of chromium, copper, zinc and nickel on aerobic and anaerobic sewage treatments. They also studied the interaction of these metals and the effects of organic loads on copper toxicity. A part of their results had been published elsewhere (5-9). Moulton and Directo (10), using the method of factorial design of experiment, studied the interactions of chromium, copper, nickel and cyanide on the activated sludge.

Also there were some reports investigating the effects of metallic wastes from electroplating or tanning industries. The staffs of R. A. Taft Sanitary Engineering Center (4) investigated the effects of chromic acid passing through the municipal treatment plants.

The present experiments were performed to give the additional informations on the effects of several metallic ions on the oxygen uptake of the activated sludge. The TC_{50} values (toxic concentration 50, the concentration of toxic substance required to obtain 50 % inhibition of the oxygen uptake of unintoxicated sludge) were determined for various metallic ions under the same condition and also detoxication effects of EDTA salt known as metallic chlating reagent were investigated.

Materials

Activated sludges were gathered from the Kagoshima sewage treatment plant. Prior to the experiment they were aerated for several days without substrates, to change the microorganisms in the activated sludge to the resting state.

The sludge concentrations were 953-4,000 ppm, the average value being 2,410 ppm.

The following salts of metal and EDTA were used.

Ag^{+}	$AgNO_3$
Hg^{2+}	$HgCl_2$
Cu^{2+}	$CuSO_4$
Ni^{2+}	$NiSO_4$
Zn^{2+}	$ZnSO_4$
Cr^{3+}	$Cr(NO_3)_3$
Cr^{6+}	CrO_3
Cd^{2+}	$CdSO_4$
Co^{2+}	$CoCl_2$
Pb^{2+}	$Pb(NO_3)_2$
EDTA	EDTA-2Na

As stock solutions, 10^{-1} molar solutions were prepared and serial 10 times dilutions were performed (10^{-2}, 10^{-3}, $10^{-4}\cdots$ mol).

Methods

The Warburg manometers were used for the measurements of oxygen uptake of the activated sludge.

The content of vessels was as follows.

Vessel	: Water	1.7 ml
	Activated sludge solution	1.0 〃
Center well	: 20% KOH solution	0.2 〃
Side arm	: Metallic ions solution	0.3 〃

For the measurement of the detoxication effect of EDTA, the vessels having two side arms were used. In this case, the content of the vessels was as follows.

Vessel	: Water	1.4 ml
	Activated sludge solution	1.0 〃
Center well	: 20% KOH solution	0.2 〃
Side arm 1	: Metallic ions solution	0.3 〃
Side arm 2	: EDTA solution	0.3 〃

The molar concentration of EDTA solutions was the same to that of metallic solutions.

The temperature of Warburg bath was 33°C. After 15 minutes of shaking, the contents of side arms were poured into the vessels. The solutions of metallic ion and EDTA were poured simultaneously. The experimental time was 6-7 hours.

Results

For example, the time-courses of oxygen uptakes of the activated sludge with an addition of Zn^{2+} salts, and with a simultaneous addition of Zn^{2+} salts and EDTA are shown in Figure 1.

The values of oxygen uptakes after 7 hours were used for calculating the TC_{50}. The values of TC_{50} were changed with the observation time. But in this experiment, the adaptation of microorganism in the activated sludge would not occur, for observation time was short and the nutrition was

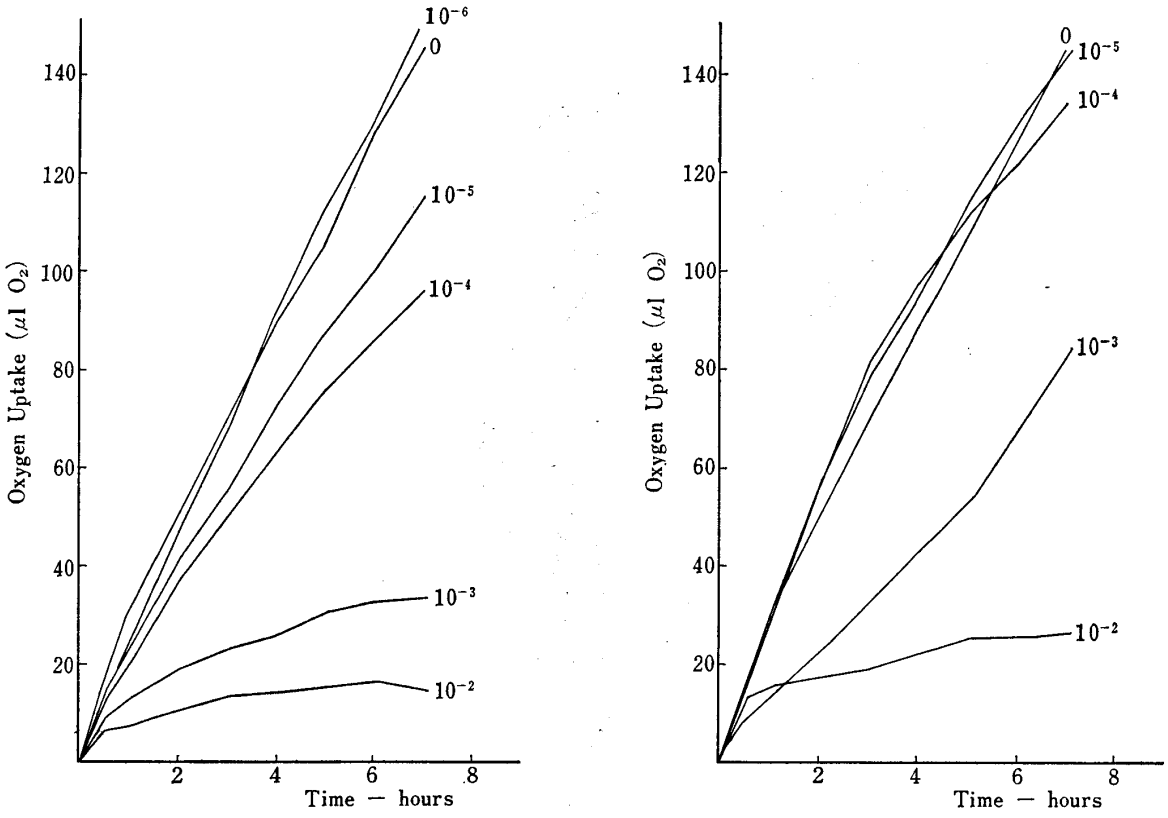

Fig. 1. Effect of $Zn^{2+}$and Zn^{2+} + EDTA on Oxygen Uptake of Activated Sludge

not added. The change in TC_{50} during the observation time was not important.

The values of TC_{50} were obtained with Doudoroff's method, which was first proposed for the calculation of TLM (Median Tolerance Limit) of fishes (13. 14). The semilogarithmic coordinate papers were used in this method. The metal concentration was plotted on logarithmic scale and the ratios of the oxygen uptake with an addition of the metallic ions to that without metallic ions were taken on arithmetic axis. The two successive points holden between 50 % of the oxygen uptake ratio were linked with straight line and the concentration of metallic ion corresponding to 50 % of the oxygen uptake ratio was obtained. This value represented TC_{50}.

This method of the determination of TC_{50} is shown in Figure 2, using the oxygen uptake after 7 th hours of Figure 1.

The experiments were repeated for various metallic salts and TC_{50} were obtained. The results are shown in Table 1. The geometric mean was used for the calculation of mean.

Also TC_{50} with simultaneous additions of metallic ions and EDTA were obtained and are shown in Table Ⅱ.

The means of TC_{50} of metallic ions with and without EDTA were compared. It is shown in Figure 3.

From the means of Table I and II the detoxication ratios (the ratio of TC_{50} with an addition of EDTA to that without EDTA) were calculated and the statistical test for significance of detoxication ratios were performed. These are shown in Table III.

From these results, the following conclusions were obtained.

1) The toxicities of metallic ions on activated sludge were decreased with the following sequence in ppm units. Cu^{2+}, Ag^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cr^{6+}, Pb^{2+}.

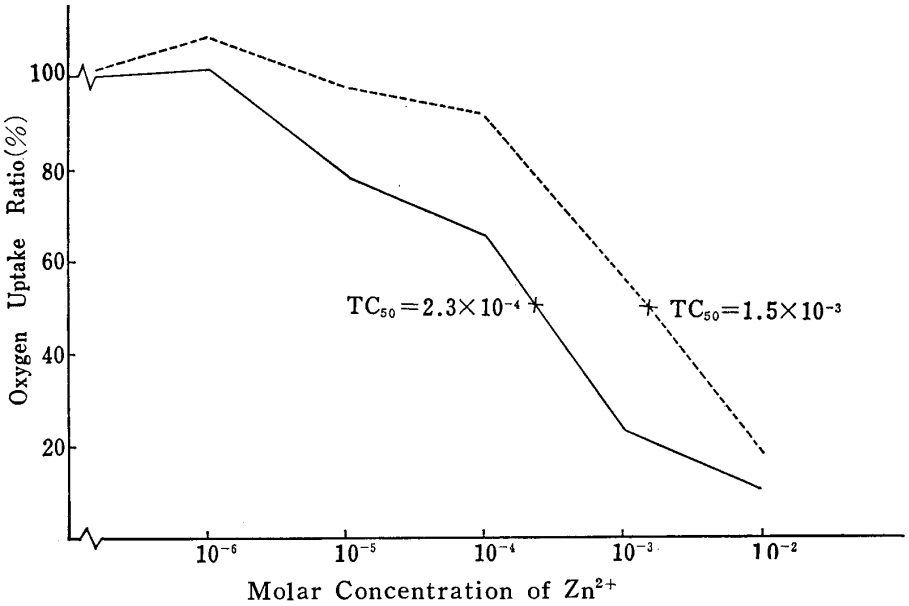

Fig. 2. Determination of TC_{50} of Zn^{2+} and Zn^{2+} + EDTA

Table I. TC_{50} of Metallic Ions

Metallic Ions	TC_{50} (Molar Concentration)	Mean*	
		Molar Concentration	ppm
Ag^{+}	1.8×10^{-5}, 3.5×10^{-5}, 3.4×10^{-5}	2.8×10^{-5}	3.0
Hg^{2+}	1.5×10^{-5}, 2.6×10^{-5}, 1.1×10^{-5}	1.7×10^{-5}	3.4
Cu^{2+}	6.8×10^{-5}, 3.6×10^{-5}, 1.6×10^{-5}	3.4×10^{-5}	2.2
Ni^{2+}	8.7×10^{-5}, 3.1×10^{-4}, 1.8×10^{-5}, 5.4×10^{-5}, 7.3×10^{-5}, 1.0×10^{-4}	7.6×10^{-5}	4.5
Cd^{2+}	6.5×10^{-5}, 8.0×10^{-5}, 1.2×10^{-4}, 6.9×10^{-5}	8.1×10^{-5}	9.1
Zn^{2+}	2.3×10^{-4}, 2.4×10^{-4}, 2.7×10^{-4}	2.5×10^{-4}	16.3
Co^{2+}	4.2×10^{-4}, 1.8×10^{-4}, 3.7×10^{-4}, 3.6×10^{-4}	3.2×10^{-4}	18.9
Cr^{3+}	5.9×10^{-4}, 2.5×10^{-4}, 4.7×10^{-4}	4.1×10^{-4}	21.3
Cr^{6+}	5.4×10^{-4}, 1.4×10^{-3}, 1.6×10^{-3}, 2.0×10^{-3}, 2.8×10^{-4}, 3.4×10^{-4}, 3.2×10^{-4}	5.0×10^{-4}	26.0
Pb^{2+}	2.7×10^{-3}, 3.2×10^{-4}, 3.4×10^{-4}, 1.2×10^{-3}, 3.8×10^{-4}, 3.2×10^{-4}, 4.5×10^{-5}	4.1×10^{-4}	85.0

* Geometric Mean

2) From the detoxication effect of EDTA, the metallic ions could be classified into 3 groups.

a) Cr^{3+}, Cr^{6+}··· the detoxication effect of EDTA could not be observed.

b) Ag^{+}, Co^{2+}··· the some detoxication effects of EDTA were noticed, but statistical analysis showed that such effects were not significant.

c) Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Hg^{2+} ··· the detoxication effects of EDTA were statistically significant.

Discussion

The values of TC_{50} were fluctuated considerably. The main cause of the fluctuation was that of the nature of activated sludge. For decreasing the effect of influent sewage, the aeration of more than 24 hours without sewage was performed prior to the experiment.

Heukelekian and Gellman (3) described that the comparison of experimental results of many rese-

Table Ⅱ. TC_{50} of Simultaneous Addition of Metallic Ions and EDTA

Metallic Ions	TC_{50} (Molar Concentration)	Mean*	
		Molar Concentration	ppm
Ag^{+}	3.9×10^{-5}, 3.0×10^{-5}, 2.5×10^{-4}, 5.3×10^{-5}, 4.9×10^{-5}	6.0×10^{-5}	6.5
Hg^{2+}	3.9×10^{-5}, 4.1×10^{-5}, 6.5×10^{-5}	4.7×10^{-5}	9.4
Cu^{2+}	1.6×10^{-3}, 2.9×10^{-3}, 2.5×10^{-4}, 3.9×10^{-4}, 6.5×10^{-5}, 3.8×10^{-4}	4.6×10^{-4}	29.2
Ni^{2+}	3.7×10^{-3}, 3.1×10^{-4}, 1.6×10^{-4}, 3.8×10^{-4}, 2.9×10^{-3}	7.3×10^{-4}	42.9
Cd^{2+}	1.5×10^{-3}, 1.9×10^{-4}, 4.0×10^{-4}, 1.5×10^{-4}, 4.0×10^{-4}	3.7×10^{-4}	41.6
Zn^{2+}	1.5×10^{-3}, 2.8×10^{-3}, 3.1×10^{-3}	2.4×10^{-3}	156.9
Co^{2+}	4.9×10^{-3}, 3.7×10^{-4}, 7.8×10^{-4}, 3.7×10^{-4}, 4.5×10^{-3}	1.2×10^{-3}	70.7
Cr^{3+}	2.4×10^{-4}, 2.9×10^{-4}, 7.8×10^{-4}	3.8×10^{-4}	19.8
Cr^{6+}	2.8×10^{-4}, 3.9×10^{-4}, 3.4×10^{-4}	3.3×10^{-4}	17.2
Pb^{2+}	3.1×10^{-3}, 2.9×10^{-3}, 1.7×10^{-3}	2.5×10^{-3}	518.0
EDTA	4.4×10^{-3}, 2.2×10^{-4}, 5.1×10^{-4}, 2.1×10^{-4}, 5.4×10^{-3}	8.9×10^{-4}	260.0**

* Geometric Means

** ppm as Ethylenediaminetetraacetic acid

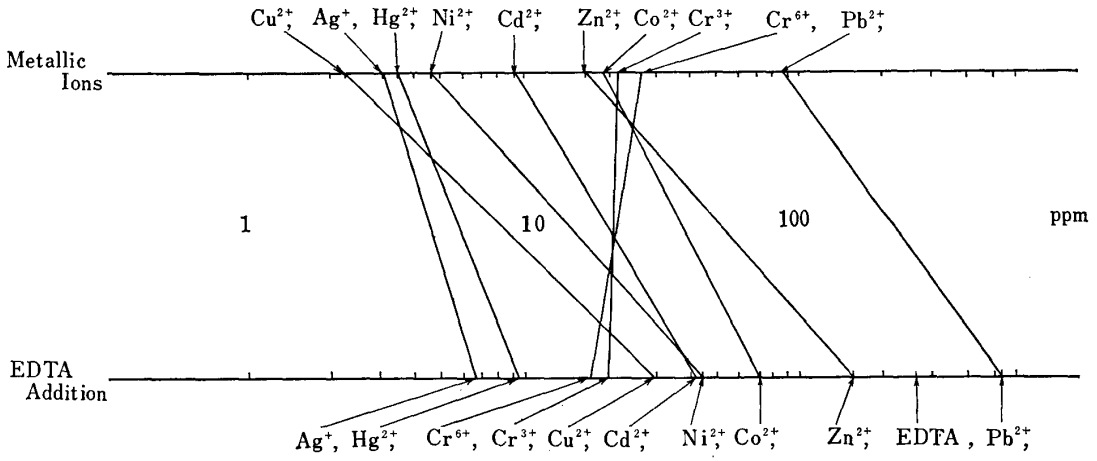

Fig. 3. TC_{50} of Metallic Ions and EDTA Addition (ppm)

arches was difficult, for the experimental methods were differed with each researcher. Ingols (15) said that the following factors had to be known to investigate the effects of toxic substance.

1) Temperature of the mixture 2) Food concentrations 3) Inorganic ions—concentrations and identity 4) Nature and identity of the organisms in the mixture 5) Previous environment of the organisms.

Though, even if the accurate comparisons were impossible, ths relative comparison of toxicity would have some meaning. In Table IV, the summarized data of several researchers are shown.

The values of Rudolfs *et al.* (1) were selected from Table IV of their review. These values were collected from the various reports till 1950. The values were smaller than those of the present results, because these values were what showed the delay or inhibition of activity.

Heukelekian and Gellman (3), using Warburg manometer, investigated the effects of metallic ions on the oxygen uptakes of sewage and activated sludge mixtures. In Table IV, the oxygen uptakes of activated sludge after 6 hours with 50 ppm of metal concentration (from Table 23 of Heukelekian and Gellman's report) were shown. They were expressed as percentage to the oxygen uptakes without

metallic ions and TC_{50} were inferred from these values. Activated sludge concentration was 2,000 ppm and temperature was 20°C. The values of TC_{50} were larger than those of the present results. The present author used the activated sludge in endogenous respiration phase, while Heukelekian and Gellman performed the experiment using the sewage-activated sludge mixed liquor. They said as to the oxidation of domestic sewage that the greater the concentration of sewage was, the smaller the effect of inhibition was. By their data, toxicity was decreased as follows. Ni^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Cr^{3+}, Co^{2+}, Cr^{6+}. This sequence nearly coincides with the present results.

R. A. Taft Sanitary Engineering Center (4), using pilot plant, investigated the effects of metal ions on activated sludge. 'Continuous dose given significant reduction in activated sludge in efficiency' and 'metallic concentration producing harmful slug for 4 hours' were shown in Table Ⅳ. The toxicity was decreased as follows; Cu^{2+}, Ni^{2+}, Zn^{2+}, Cr^{6+}. This sequeuce also coincides with the present results.

Dawson and Jenkins (16), using Warburg manometers, said that

Table Ⅲ. Detoxication Ratios of EDTA on Metallic Ions

Metallic Ions	Detoxication Ratios
Ag^{+}	2.2
Hg^{2+}	2.8*
Cu^{2+}	13.3*
Ni^{2+}	9.5*
Cd^{2+}	4.6*
Zn^{2+}	9.6**
Co^{2+}	3.7
Cr^{3+}	0.9
Cr^{6+}	0.7
Pb^{2+}	6.1*

Detoxication Ratios $= \frac{TC_{50} \text{ when EDTA added}}{TC_{50} \text{ when no EDTA added}}$

* Significant under 5% risk

** Significant under 1% risk

Table Ⅳ. Literatures of the Effect of Metallic Ions on Activated Sludge

Metallic Ions	Rudolfs et al[1] Concentration Showing the Retardation or Inhibition of Activities	Heukelekian and Gellman[3] (6 hrs) Oxygen uptake at 50ppm of Metal	Heukelekian and Gellman[3] (6 hrs) TC_{50} (Estimated)	R. A. Taft Sani. Engi. Center Continuous Dose Given Significant Reduction in Efficiency	R. A. Taft Sani. Engi. Center Concentration Producing Harmful Slug (4 hrs)
	ppm	%	ppm	ppm	ppm
Cu^{2+}	1	41	25	1	75
Ni^{2+}	1~3	35	10~25	1~2.5	50~200
Cd^{2+}		43	25~50		
Zn^{2+}		50	50	5~10	160
Co^{2+}		63	>100		
Cr^{3+}	2	72	50~100		
Cr^{6+}		65	>100	10	>500
Pb^{2+}	0.1				

Zn^{2+} was the most toxic cationic ion to activated sludge, and the toxicity of metallic ions was varied with the coexistence of other metallic ions or the kinds of metallic ions compounds.

The representative results for the effect of metallic ions on BOD tests were summaried in Table V.

TC_{50} values of metallic ions would be different between activated sludge and BOD test. Research Committee (17) described that the effect of metallic ions on BOD value was not important for sewage treatment processes, but for river pollution. However, the BOD reaction and oxygen uptake of activated sludge were both based on the respiration of aerobic microorganisms, so comparison of toxicity might be significant.

Hiro (18), using the diluted river water and sewage as the substrate, reported that the inhibition

Table V. Literatures of the Effect of Metallic Ions on BOD Test

Metallic Ions	Hiro[18]		Research Comm.[17]	Heukelekian and Gellman[3]		Hermann[19]	Sheets[20]
	Inhibition Clearly	Inhibition Completely	TC_{50}	1 day	5 days	TC_{50}	TC**
	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Ag^{+}							0.3
Hg^{2+}	0.5	10	$HgCl_2$*−0.32			$HgCl_2$*-0.61	
Cu^{2+}	0.5	20		3	35	$CuSO_4$−21	0.4
Ni^{2+}	5	50		3	15		16.0
Cd^{2+}	5	50		10	55	142	
Zn^{2+}	10～20	40 % oxygen uptake at 50 ppm	68 % oxygen uptake at 10 ppm	30	62.5		(920)
Co^{2+}	5	50		5	25	$CoCl_2$*−64	
Cr^{3+}	2.5～5.0	20	6.0	17.5	62.5	17	0.23(0.18)
Cr^{6+}	10	50	70 % oxygen uptake at 10 ppm	>100	>100		4.0 (0. 9)
Pb^{2+}	50						

* ppm as metallic salts

** the values in parentheses were obtained using the non-buffered dilution solution

effect of metals was decreasted as follows, Hg, Cu, Cr^{3+}, Ni, Cd, Co, Cr^{6+}, Zn, Al, Pb, Mn. This sequence resembled to the present results except that the toxicity of Cr^{3+} was larger than those of Ni and Cd.

The values of Research Committee (17) were collected from the results of many laboratories. The toxicity of Hg was specially larger and toxicity decreased with Cr^{3+}, Zn^{2+}, Cr^{6+} sequence.

Heukelekian and Gellman (3), using the Warburg manometers, investigated the toxicity was decreased as follows ; Ni^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Cr^{3+}, Zn^{2+}, Cr^{6+}. They also described that the metallic ion concentrations required to produce a particular degree of oxidation were increased as the experimental time proceeded.

Hermann (19) performed the experiment using glucose and polypeptone as the substrate. According to his results, the toxicity of $HgCl_2$ was 30 times larger than that of $CuSO_4$. TC_{50} of Cd^{2+} was 142 ppm and this value was relatively large.

Sheets (20) used two kinds of dilution solution, that is, with and without buffer action. In Table V, the values of TC_{50} for the same compounds with present author's were selected from his report. (only as Cr salts, $CrCl_3$ was used in his report). The toxicities of Cr^{3+} and Cr^{6+} were higher than the values of other reports.

For the detoxication effect with EDTA, Hiro (18) classified the metals into two groups.

1) The inhibition effect was high and the detoxication effect with EDTA was low…Hg, Cr.

2) The inhibition effect existed and the detoxication effect with EDTA was observed…Cu, Co, Ni, Zn, Cd, Pb.

This result agreed generally with the present results.

Morgan and Lackey (21) observed that the toxicities of Cu and Cr^{3+} on the BOD test of sewage decreased with an addition of EDTA and described that the toxic effect of metallic ions on the BOD test or biological treatment could be removed with an addition of EDTA.

The chelate formation constants of EDTA with metal are decreased with the following sequence. The ionic strength is 0.1 and the temperature is 20°C (22).

$$Hg > Cu, Ni, Pb > Zn, Cd, Co > Ag$$

Except Hg, this sequence was nearly consistent with the sequence of the detoxication ratios of author's.

Salle (23) described that the antiseptics effect of metallic ions was not very large and except Hg and Ag the effect of metals was as that of astriction.

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