The hydrogen-ion concentrations in Experiments 1–15 lie between $10^{-4.42}$ and $10^{-4.58}$. The experiment given below gives an idea of the effect of a small variation in the acidity. This experiment is a duplicate of Experiment 13, Table I. The velocity coefficients of Experiment 13 ($C_{H^+} = 10^{-4.52}$) are given in the last column for comparison. A comparison of the two shows that the difference in hydrogen-ion concentration makes little difference in the velocity of the reaction.

20 g. Sugar and 2 cc. Invertase A per 100 cc. $C_{H}^{+} = 10^{-4.38}$.

Time.	Observed rotation.	Per cent. inversion.	$1/t \log 1/1 - x'$.	Vel. coeff. Expt. No. 13.
0	24.15	.....	.....	.....
55	21.07	9.82	0.00082	0.00081
115	17.88	19.99	0.00084	0.00086
240	11.69	39.72	0.00092	0.00093
359	6.69	55.66	0.00098	0.00101
710	—2.01	83.39	0.00110	0.00110
1340	—6.00	96.11	0.00105	0.00104
	—7.22	.....	.....	.....

NEW YORK, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MONTANA.]

COPPER IN THE FLORA OF A COPPER-TAILING REGION.

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Received February 12, 1917.

During the past decade extensive and valuable information has contributed much to the knowledge of the effect of smelter smoke and dust on the plants and stock living in adjacent regions. Another waste product of the smelters, the tailings, has, however, received little attention. This paper notes some effects caused by the tailings of a large copper smelter on the native flora of the vicinity.

This smelter is that located near Anaconda, Montana. It is situated at the base of a mountain ridge at the Southwest border of Deer Lodge Valley, which is about forty miles long and from four to six miles wide. The tailings from the smelter are emptied into Warm Springs Creek, find their way across the valley in many small streams and ultimately flow into the Missoula River. Along the way the heavier metallic particles of the material are gradually deposited while many other streams join the main waterway of the region. But even at Missoula, a distance of about one hundred miles, the water is very turbid from the tailings of the smelter above.

A trip through the region affected by the tailings presents a very interesting picture. Before their advent the soil supported the characteristic flora of this district which is still seen outside the tailing areas and across the river on the other side of the valley. There flourishing willows line the little streams while grasses of various kinds, the wild rose, and clover among other things grow abundantly. Occasionally fields of alfalfa are seen, once on farms since purchased by the smelter. The western sage is also found and many clumps of tall mullein—altogether a typical mountain valley. In contrast, among the tailings the willows in places stand black and dead for thousands of yards at a stretch while at others they have an unhealthy appearance, or more infrequently seem hale and green. Over extensive areas no plant life at all is to be seen. The grasses when they are found are usually sparse although some varieties make a good stand during the season. Sage, clover and mullein seem to have entirely deserted the valley and among the larger shrubs only the wild rose appears to flourish.

Other changes are more striking. The soil is gradually covered by the tailing solids which impart to it a variety of colors, in some cases gray, in others yellow or bright red from ferric oxide. For miles along the streams where the water is evaporated away the ground is encrusted with masses of bright blue and green deposits, often crystalline. This is especially true where the crystals have had a chance to form on grass blades, stems or even on barbed wire. These substances which have only been roughly examined appear to be: the blue, a basic copper sulfate, and the green a mixture of copper and iron sulfates. No carbonates of copper were found in any samples. The water in many of the rivulets is decidedly acid with sulfuric acid while the rocks in the bed of the streams are mostly changed by metathetical reactions into velvety pebbles of various shades of green, the color again being due to compounds of copper. Even the bones of perished stock, instead of being bleached, are dyed a vivid green.

Samples of the flora and soils from this region were collected in the early part of October, 1914, after two weeks of rather constant rain. The season had been such that it insured good crops and a luxuriant vegetation.

The plants had been well washed by the autumnal rains and no dust had been blown about for some time. Nevertheless, the specimens were carefully sorted and washed for several hours in running water to obviate as much as possible the danger of mistaking substances in the dust adhering to the surface of the plants for those actually in the tissues. By repeatedly turning the samples in the stream of water all loose particles, dirt and dust were carried away. After washing, the samples were dried in a warm room. In all cases the results obtained are from such air dried specimens.

For analysis the tissues were destroyed by a modification of the Chittenden-Donaldson method of decomposition of animal tissue made applicable to plant tissue by Swain and Harkins[21] in their extensive work on arsenic in the vegetation of this same valley. Large samples were used,

TABLE I.

No.	Sample.		Copper.	Arsenic.	Antimony.	Zinc.	Iron.
1.	Willow. *Salix fluviatilis* No. 1..........	stems and leaves	fair	small	trace	good	fair
2.	Willow. *Salix fluviatilis* No. 2..........	dead branches	good	....	none	large	good
3.	Willow *Salix fluviatilis* No. 3..........	stems and leaves	fair	small	trace	good	fair
4.	Willow. *Salix fluviatilis* No. 4..........	dead branches	good	good	fair	large	large
5.	Willow. *Salix fluviatilis* No. 5..........	leaves	fair	large	good	fair	trace
6.	Alfalfa..............................	stems and leaves	fair	good	fair	fair	fair
7.	Red Clover..........................	stems and leaves	good	good	fair	good	good
8.	Plantain. *Plantago major*...............	stems and leaves	large	good	fair	large	good
9.	Plantain. *Plantago major*...............	roots	fair	....	none	fair	fair
10.	Grass. *Agropyron lanceolatum* No. 1....	blades	good	fair	trace	good	good
11.	Grass. *Paspalum* No. 2....	blades	small	fair	trace	fair	fair
12.	Grass. *Paspalum* No. 3....	blades	v. lge.	trace	trace	fair	fair
13.	*Dasiophora fruticosa*....................	whole plant	good	trace	none	fair	large
14.	Wild Rose. *Rosa Woodsii*..............	whole plant	fair	fair	trace	trace	trace
15.	Horsetail. *Equisetum variegatum*........	whole plant	fair	fair	trace	fair	trace

in most cases about 15 g. The residue from decomposition was repeatedly extracted with dilute sulfuric acid (5–6 cc. 6 N acid in 100 cc. of water) to which a drop or two of nitric acid had been added to dissolve any oxides that might otherwise have been left undissolved. Acid of this concentration gave a solution affording a good precipitation of copper and arsenic groups by hydrogen sulfide. When the amounts of metals obtained were small careful confirmatory tests were made in all cases.

In Table I the results of representative qualitative analyses are gathered together. The table shows the relative amounts present.

These plants were collected as follows:

Willow 1.—This sample was taken from a large stand of trees along the bank of Silver Bow Creek which flows from Butte and carries much of the mine waters from that mining camp, the water being of a grayish brown color. These willows appeared in part healthy and this sample is from such a tree. A large amount was taken from various parts.

Willow 2.—Was a dead willow growing on the same soil as the preceding. Since the bark was still held firmly to the dead wood this willow must have been killed but a short time previously.

Willow 3.—Was collected in the same region as the others. The trees here were some distance from the stream; but the soil had been overflowed many times so that it was covered with tailing solids. Samples of soil were collected for these three specimens.

Willow 4.—Was collected from a great clump of dead trees standing some distance down the stream from the preceding. They had once been vigorous, having attained a height of fifteen feet. The bark was not so firmly adherent to the wood in this case but the trees had been dead for a comparatively short period.

Willow 5.—This willow was growing among many others along a stream coming from Anaconda. The water was distinctly acid and the rocks lying about were colored green. The tailings had not been deposited here to any extent but were running down a stream a few hundred feet away. The trees did not appear vigorous.

Alfalfa.—This plant was found growing on deserted farm land. The field had the appearance of having been flooded by water carrying tailings. Only a few clumps of alfalfa were left in the slightly elevated spots of the field while no grass at all was found at this place. A soil sample was taken here.

Clover.—This was found in large patches in the same field as the alfalfa. These plants appeared to have been suddenly killed while in full bloom.

Plantain.—The specimen was taken from a growth of this plant found along a stream. The thick and fleshy roots seemed to have been corroded by the acid tailings.

Grass 1.—This was apparently uninjured although growing in a gray metallic deposit of tailings many feet thick.

Grass 2.—A different species found near Grass 1.

Grass 3.—Samples were collected from a clump of grass that enclosed a large lump of the green and blue substances mentioned above. The lower part of the blades and the exposed roots had become heavily encrusted with copper salts crystallized from the tailings. This and all the grass nearby was badly injured.

Dasiophora fruticosa.—This bush-like plant was found growing along the bank of a waterway in the deposited tailings. It was apparently in the best of condition and bore a wealth of its small yellow flowers. For a radius of many yards from this plant no others were growing and this was true for a considerable area along this stream. Nevertheless, the Dasiophora was found everywhere growing under adverse conditions.

Wild Rose.—This plant, too, was found growing luxuriantly in the most abnormal looking soils where no other plant life was able to maintain itself.

Horsetail.—Another plant apparently unaffected by the unusual environment of the tailing deposits. The sample analyzed was growing near the grasses listed above.

The soil in which Willows 1 and 2 were found contained fairly large amounts of copper and zinc with a much smaller amount of arsenic. These were found in a hot water solution of 10 g. of soil. The water solution was acid to litmus.

Analyzed in the same way the soil near Willow 3 contained the same metals but in greater amount than the last sample and besides was more acid.

Samples of soil in which the alfalfa was found failed to give positive evidence of copper or arsenic but did contain zinc. The same was true of soil from about the clover roots and near the plantain.

A water solution of the tailings deposits in which Grass 1, the *Dasiophora* and the *Equisetum* were growing so well contained arsenic, antimony, copper, iron, zinc, thallium and free sulfuric acid.

The rose bushes were growing in clumps on bright red deposits which yielded to water extractions arsenic, antimony, copper, large amounts of iron and sulfate and smaller amounts of zinc, cadmium, thallium, with traces of lead. Acid solutions of all these soils gave much larger amounts of all the metallic constituents and small amounts, in the last two cases, of selenium, tellurium, manganese, magnesium and aluminium.

All the samples had been well leached out by the two weeks of rainy weather immediately preceding their collection.

All the plant samples listed above were further analyzed quantitatively for copper. The tissues were decomposed in the same manner as before but the residue was dissolved in dilute nitric acid, the excess of which was then evaporated away. The copper was deposited electrolytically in platinum crucibles from a faintly acid solution carrying a small amount of urea. The various parts of the willows, the leaves, bark and wood, were analyzed separately. Table II shows the results.

TABLE II.

No.	Sample.	Copper content in %.	Copper content in Pts. per 10^6.
1.	Willow 1. Growing leaves	0.0192	192
2.	Willow 1. Growing bark	0.0207	207
3.	Willow 1. Growing wood	0.0046	46
4.	Willow 2. Dead bark	0.1760	1760
5.	Willow 2. Dead wood	0.0426	426
6.	Willow 3. Growing leaves	0.1560	1560
7.	Willow 4. Dead bark	0.1426	1426
8.	Willow 4. Dead wood	0.0644	644
9.	Willow 5. Growing leaves	0.0139	139
10.	Alfalfa. Stem and leaves	0.0252	252
11.	Clover. Dead	0.0655	655
12.	Plantain. Leaves and stems	0.6210	6210
13.	Plantain. Root	0.0260	260
14.	Grass 1	0.4820	4820
15.	Grass 2	0.1052	1052
16.	*Dasiophora fruticosa.* Bark	0.2510	2510
17.	*Dasiophora fruticosa.* Wood	0.0111	111
18.	Rosebush. Leaves and fruit	0.0145	145
19.	Rosebush. Bark and wood	0.0909	909
20.	Horsetail	0.0296	296

Some of these figures are notably high, especially those for the plantain leaves, Grass 1 and the bark of *Dasiophora.* Swain and Harkins[20] in many samples of grass and hay grown in this same region found figures ranging from 11 to 1800 parts per million although half of the samples range only from 100 to 400 parts. Frankforter[6] found in a dead oak quantities as high as 39.5 parts per million. MacDougal[13] found in another oak (*Quercus macrocarpa*) nearly 500 parts per million of copper. In both these specimens the copper could be seen as reddish particles. MacDougal further states that plants grown in ordinary soils may carry 30 parts per million of this metal. Heckel[7] found 30 and 560 parts of copper per million in samples of *Polycarpaea spirostylis.* Other plants growing on soils rich in copper contained as much as 500 parts. The ash from seeds of Quassia Gabonensis was found to contain 0.698% of copper which was mostly in the seed coats since the ash of the kernel minus the seed coat contained only 0.254%. Lehmann[11] found as high as 560 mg. per kilo in plants grown in coppery soils and as much as 30 mg. in garden vegetables. Dieulafait[5] showed plants growing on various soils to contain appreciable amounts of copper.

Swain and Harkins show most of the copper content of their samples to come not from the tissues of the plant but from its surface where it was held in the form of finely divided dust from the smelter smoke which filled the valley. Plants grown in soils of the valley but out of range of the smoke contained much less. In the above samples the bark of Willows 1, 2 and 4 and the bark of the *Dasiophora* contain more copper than the wood, in three cases the amounts being very disproportionate. The plantain leaves show the highest amount of all. These big leaves being close to the ground offer a large surface for the adherence of copper in the soil. This is true of the grasess as well and it may be impossible to remove all the dust from any of the samples even by the prolonged washing described above. The amounts found in the wood which must have been reached by absorption are not much above those reported by other observers and are the lowest of all the values found. However, the bark of trees and shrubs is apparently the natural storehouse for foreign substances which get into the plant and where the trees are forced to take up copper for some years the values in the bark and perhaps other parts may well be higher than those found for annuals such as grass. Lehmann[11] reports 36.3 parts of copper in the bark of a wild plum tree and only 2 in the wood while the figures for a juniper are 150 and 36 parts, respectively.

The question of the toxicity of copper to plants has been much debated. That it is in general extremely poisonous to the lower organisms is of course not doubted. Naegeli[11] and Israel and Klingmann[8] demonstrated the great sensitiveness of bacteria and spirogyra to this substance.which has, since then, been made of much economic importance, Moore and Kellermann,[15] Kraemer,[10] Jones,[9] Thomas.[22]

With its effect on the higher plants, on the contrary, investigators are not in harmony. Deberain and Demoussy[3] found slight traces of copper to inhibit germination of certain seeds but later Demoussy[4] thought the ion to be without practical effect. Coupin[1] from his experiments on the poisonous effects of cupric salts on young wheat plants concluded that the use of solutions of these compounds as a germicide is attended with considerable risk. Frankforter[6] thought his oak tree might have died from the copper it contained. Richter[17] found that extremely dilute solutions of copper sulfate inhibited the growth of *Aspergillus niger* while Montemartini[14] claims that the same solutions exert a strong stimulating action on other plants. Lehmann[12] at first considered copper to decidedly injure plants but later from a number of other studies came to the conclusion, as did Tschirch[23] that copper could not be very toxic to the higher plants.

The role that copper plays in the disease and death of plants in our tailing region is also in doubt. It is true that the wood of two dead willows contained twice and thrice as much copper as the wood of a living tree growing close beside the first dead one; also the dead clover in the same field as the still growing alfalfa contained over twice as much copper. But the vigorous roses, *Dasiophora* and horsetail also contain fair amounts of the metal. These plants may possibly have more resistance than others or more adaptability. The fact that certain plants noted above have disappeared from the tailings area points in this direction. The freely respiring plants using a very large quantity of water in the growing season seem especially sensitive to these conditions; the willows appear to be waging a losing fight while many other plants are still successful in maintaining themselves.

Other factors may be more potent than the copper content. Practically all these plants contained arsenic—some in large amount—many contained antimony, all contained zinc and some unusually large amounts of iron. Just how these metals may affect plants is debated. Again the free sulfuric acid in the water may play a large part in killing the vegetation. Many plants normal above the surface were found to have charred, blackened and decomposed roots.

Copper and arsenic are not normal constituents of the above plants. This is shown by analyzing specimens of willow, rosebush, horsetail and grass from the vicinity of Missoula where the soils contain only traces of copper. None of the plants gave any indication of containing this metal; nor did others, such as tumble weed and mullein. The water of the Missoula River contained but a very small amount of copper in the residue from 5 liters although this stream receives the copper waste from a smelter a hundred miles away. A stream flowing into the river at Missoula gave no test for copper in the residue from 15 liters of water. These tests furnish a blank test upon the reagents used in testing for copper in the tailing plants.

The presence of antimony and zinc in the plants suggested the examination of a number of organs from cattle and horses once living in the valley and which had been analyzed by Swain and Harkins[21] for arsenic. All of them contained zinc, a number antimony, others aluminium and a few thallium. In view of the toxicity of those substances—zinc,[18] aluminium,[24] thallium[19]—one may well speculate over their combined effect on the life of the region.

Summary.

1. Plants growing in a copper tailing region were found to contain appreciable amounts of copper, arsenic, antimony and zinc.

2. The amounts of copper ranged from 0.0046% to 0.621%, being larger in dead than in living tissue and greater in the bark than in other parts of the plant.

3. Many plants are unable to adapt themselves to this novel factor of environment while others flourish in spite of it, thus showing a decided selective activity.

In conclusion it is a pleasure to thank Dr. Kirkwood, Professor of Botany in the University of Montana, for his helpful suggestions.

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