

# Effect of Metal Ions on ALP Activities in Raw and Pasteurized Milk

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**Abstract:** This study was aimed to examine the activity of alkaline phosphatase enzyme in pasteurized milk upon addition of different metal ions. Here we have taken 5 sets of 5 metal ions with different concentration to check out the effect of metal ions on the reactivation of alkaline phosphatase enzyme into the pasteurized milk. It was observed that certain metal ions are exhibiting the reactivation of alkaline phosphatase enzyme into the pasteurized milk and shows AP test positive while certain metal ion is highly inhibiting the reactivation of alkaline phosphatase enzyme and shows the AP test negative in the pasteurized milk.

**Keywords:** Alkaline phosphatase enzyme, Pasteurized milk, Metal ions, Alkaline phosphatase enzyme reactivation.

## INTRODUCTION

As a leading producer of the milk, Indian dairy sectors are growing widely. Now they focus mainly on the quality of the milk and milk products. In any dairy industry the basic test for identifying the quality of the raw milk are the organoleptic, %Fat, %SNF, %acidity, COB and MBRT while for the pasteurized milk the basic tests are ALP activity test, MBRT, %Fat, %SNF are there. These tests are popular all around the globe to perform. AP test helps in knowing the activity of alkaline phosphatase enzyme in the milk. Now as farming system of India and its different region the quality of milk will differ from each other like there may be presence of heavy metals in the raw milk which can affect the health of the humans. It comes to the milk via fodder. In many region of the India the presence of heavy metal ions are there. It does not only affect the health of the humans but also it affects the state of the soil and environment. The metals ions come from the fodder. Animals consume this fodder and when we secrete the milk metals comes into the milk. The presence of metal ions causes damage to the human body like it directly effects on the liver and kidney and causes other diseases also.

## REAGENTS AND EQUIPMENT'S REQUIRED

Water bath (D.K scientific technologies), Sodium carbonate (Merck specialities private limited, 1.93611.0521), Sodium bicarbonate (RFCL limited, 50680), PNPP (SISCO research laboratories private limited, 88485), Zinc (S D fine chem. Limited, 20297 k05), Silver (Merck specialities private limited, 61755900251730), Copper (Samir tech chem. Industry, 30310), Cobalt (Glaxo smithkline pharmaceuticals, 59876209), Calcium (Samir tech chem. Private limited, 910443), Manganese.

## PROCEDURE

The study was aimed to metal ions influence on the quality of milk as ALP activity. Total 7 metal ions were tested for the activity of ALP on the raw and pasteurized milk. They were kept in the standard calibrated water bath. The 5 metals are Zinc (S D fine chem. Limited, 20297 k05), Copper (Samir tech chem. Industry, 30310), Cobalt (Glaxo smith Kline pharmaceuticals, 59876209), Calcium (Samir tech chem. Private limited, 910443) and Manganese. The samples were analysed at the interval of 30 minutes with standard methodology. The ALP activity was checked by 1ml raw and pasteurized milk sample in presterilized test tube (Borosil, 9830) followed by addition of different strengths of solutions like 0.%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0% of each one of the five metals. After mixing it thoroughly with the raw and pasteurized milk 5ml of AP buffer PNPP (SISCO research laboratories private limited, 88485) which was earlier prepared in the buffer containing 1.5g of Sodium bicarbonate (RFCL limited, 50680) and 3.5g of Sodium carbonate (Merck specialities private limited, 1.93611.0521) and adjusted its pH value 10.2. The ALP activities were checked at the 37°C temperature by incubating test samples in standard water bath (D.K scientific technologies) which was previously calibrated before study. The yellow colour appearance indicated the presence of alkaline phosphatase enzyme which means the test is positive. All results as per the action plan were documented properly.

## RESULTS

Here we have done the Alkaline phosphatase activity test on 5 different metals so for that to know the metal activity we have to know the at what time the alkaline phosphatase enzyme gets activate at what time. Here we have checked the time required in the pasteurized milk to reactivate the alkaline phosphatase enzyme which is 6 hours. So from this we can tell that at 6 hours the alkaline phosphatase enzyme gets activated in the milk.

Table 1. Control sample readings

PCM Control	
Time (hrs.)	ALP activity +ve/-ve
0	Negative
1	Negative
2	Negative
3	Negative
4	Negative
5	Negative
6	Positive
7	Positive
8	Positive
9	Positive
10	Positive
11	Positive
12	Positive
13	Positive
14	Positive
15	Positive
16	Positive
17	Positive
18	Positive
19	Positive
20	Positive
21	Positive
22	Positive
23	Positive
24	Positive

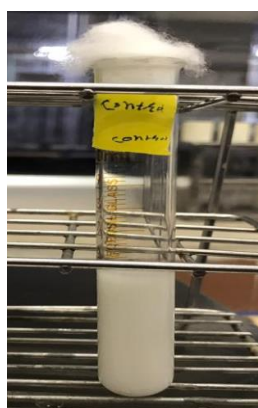


Figure 1. Reading of control samples at 0 hour

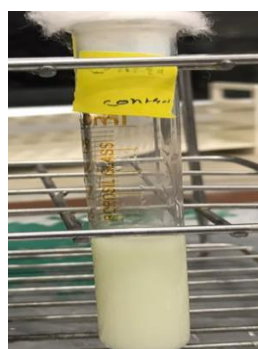


Figure 2. Reading of control samples at 6 hour

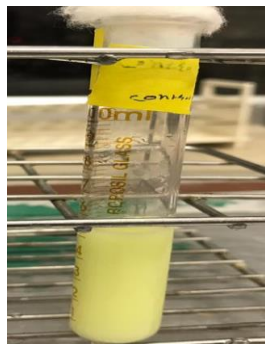


Figure 3. Reading of control samples at 12 hour

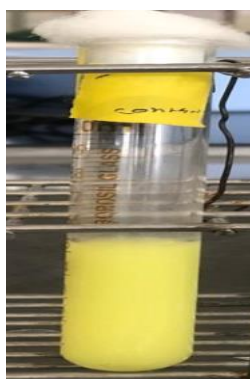


Figure 4. Reading of control samples at 24 hour

Copper (Samir tech chem. Industry, 30310)

We have checked the activity of metals on alkaline phosphatase enzyme reactivation. In this we have made different concentration of metal solution of 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% by this different concentration we can check the time required for the reactivation of the alkaline phosphatase enzyme in the pasteurized, milk. We have checked the metal effect on alkaline phosphatase enzyme in pasteurized milk for 24 hours in a water bath (D.K scientific technologies) at 36°C. In this we have checked it by using five different metals one of that is copper (Samir tech chem. Industry, 30310). Here at 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0% concentration the alkaline phosphatase enzyme does not reactivate in the pasteurized milk for 24 hour.

Table 2. Readings of Copper test samples

	Copper solution concentration effect on ALP test					
Time (Hrs)	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
0	Negative	Negative	Negative	Negative	Negative	Negative
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative	Negative	Negative
7	Negative	Negative	Negative	Negative	Negative	Negative
8	Negative	Negative	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative	Negative	Negative
14	Negative	Negative	Negative	Negative	Negative	Negative
15	Negative	Negative	Negative	Negative	Negative	Negative
16	Negative	Negative	Negative	Negative	Negative	Negative
17	Negative	Negative	Negative	Negative	Negative	Negative
18	Negative	Negative	Negative	Negative	Negative	Negative
19	Negative	Negative	Negative	Negative	Negative	Negative
20	Negative	Negative	Negative	Negative	Negative	Negative
21	Negative	Negative	Negative	Negative	Negative	Negative
22	Negative	Negative	Negative	Negative	Negative	Negative
23	Negative	Negative	Negative	Negative	Negative	Negative
24	Negative	Negative	Negative	Negative	Negative	Negative



Figure 5. Reading of copper test samples at 0 hour



Figure 6. Reading of copper test samples at 8 hour



Figure 7. Reading of copper test samples at 16 hour



Figure 8. Reading of copper test samples at 24 hour

Zinc (S D fine chem. Limited, 20297 k05)

We have checked the activity of metals on alkaline phosphatase enzyme reactivation. In this we have made different concentration of metal solution of 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% by this different concentration we can check the time required for the reactivation of the alkaline phosphatase enzyme in the pasteurized, milk. We have checked the metal effect on alkaline phosphatase enzyme in pasteurized milk for 24 hours in a water bath (D.K scientific technologies) at 36°C. In this we have checked it by using five different metals one of that is zinc(S D fine chem. Limited, 20297 k05). Here at different time the alkaline phosphatase enzyme gets activate in the pasteurized milk which is shown in the below table,

Table 3. Readings of zinc test samples

Time (Hrs)	Zinc solution concentration effect on ALP test					
	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
0	Negative	Negative	Negative	Negative	Negative	Negative
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative	Negative	Negative
7	Positive	Negative	Negative	Negative	Negative	Negative
8	Positive	Positive	Negative	Negative	Negative	Negative
9	Positive	Positive	Positive	Negative	Negative	Negative
10	Positive	Positive	Positive	Negative	Negative	Negative
11	Positive	Positive	Positive	Positive	Negative	Negative
12	Positive	Positive	Positive	Positive	Positive	Negative
13	Positive	Positive	Positive	Positive	Positive	Negative
14	Positive	Positive	Positive	Positive	Positive	Positive
15	Positive	Positive	Positive	Positive	Positive	Positive
16	Positive	Positive	Positive	Positive	Positive	Positive
17	Positive	Positive	Positive	Positive	Positive	Positive
18	Positive	Positive	Positive	Positive	Positive	Positive
19	Positive	Positive	Positive	Positive	Positive	Positive
20	Positive	Positive	Positive	Positive	Positive	Positive
21	Positive	Positive	Positive	Positive	Positive	Positive
22	Positive	Positive	Positive	Positive	Positive	Positive
23	Positive	Positive	Positive	Positive	Positive	Positive
24	Positive	Positive	Positive	Positive	Positive	Positive



Figure 9. Reading of zinc test samples at 0 hour



Figure 10. Reading of zinc test samples at 7 hour



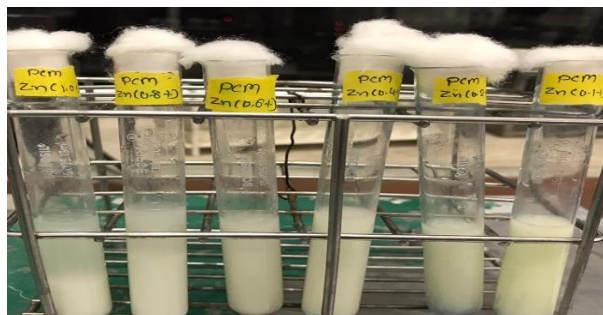


Figure 11. Reading of zinc test samples at 8 hour



Figure 12. Reading of zinc test samples at 9 hour



Figure 13. Reading of zinc test samples at 11 hour



Figure 14. Reading of zinc test samples at 12 hour

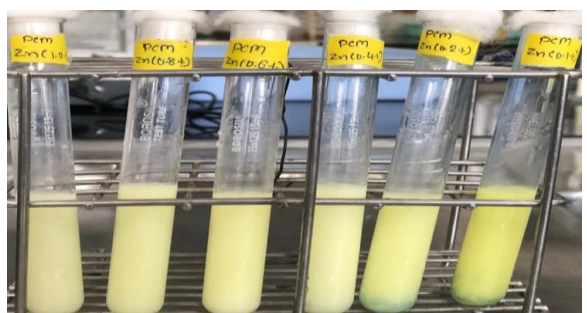


Figure 15. Reading of zinc test samples at 14 hour

## Manganese

We have checked the activity of metals on alkaline phosphatase enzyme reactivation. In this we have made different concentration of metal solution of 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% by this different concentration we can check the time required for the reactivation of the alkaline phosphatase enzyme in the pasteurized, milk. We have checked the metal effect on alkaline phosphatase enzyme in pasteurized milk for 24 hours in a water bath (D.K scientific technologies) at 36°C. In this we have checked it by using five different metals one of that is manganese. Here at different time the alkaline phosphatase enzyme gets activate in the pasteurized milk which is shown in the below table,

Table 4. Readings of manganese test samples

Time (Hrs)	Manganese solution concentration effect on ALP test					
	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
0	Negative	Negative	Negative	Negative	Negative	Negative
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative	Negative	Negative
7	Negative	Negative	Negative	Negative	Negative	Negative
8	Negative	Negative	Negative	Negative	Negative	Negative
9	Positive	Positive	Negative	Negative	Negative	Negative
10	Positive	Positive	Negative	Negative	Negative	Negative
11	Positive	Positive	Positive	Negative	Negative	Negative
12	Positive	Positive	Positive	Positive	Positive	Negative
13	Positive	Positive	Positive	Positive	Positive	Negative
14	Positive	Positive	Positive	Positive	Positive	Positive
15	Positive	Positive	Positive	Positive	Positive	Positive
16	Positive	Positive	Positive	Positive	Positive	Positive
17	Positive	Positive	Positive	Positive	Positive	Positive
18	Positive	Positive	Positive	Positive	Positive	Positive
19	Positive	Positive	Positive	Positive	Positive	Positive
20	Positive	Positive	Positive	Positive	Positive	Positive
21	Positive	Positive	Positive	Positive	Positive	Positive
22	Positive	Positive	Positive	Positive	Positive	Positive
23	Positive	Positive	Positive	Positive	Positive	Positive
24	Positive	Positive	Positive	Positive	Positive	Positive



Figure 16. Readings of manganese test samples at 0 hour



Figure 17. Readings of manganese test samples at 9 hour

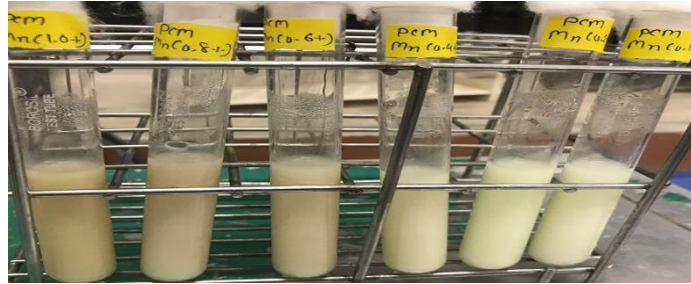


Figure 18. Readings of manganese test samples at 11 hour

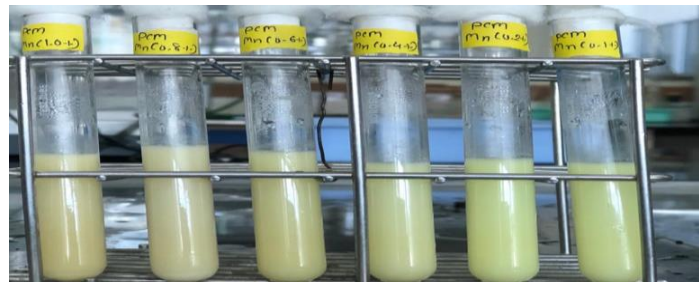


Figure 19. Readings of manganese test samples at 12 hour

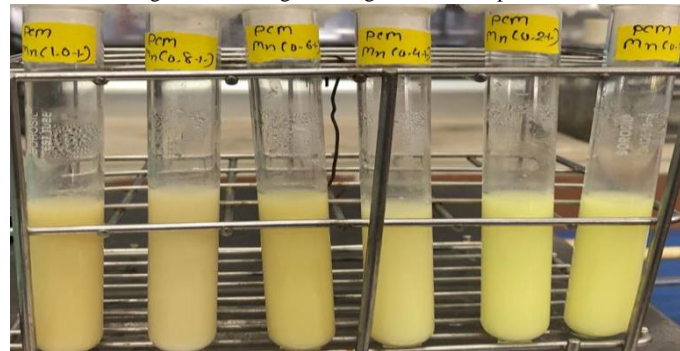


Figure 19. Readings of manganese test samples at 14 hour

Cobalt (Glaxo smithkline pharmaceuticals, 59876209)

We have checked the activity of metals on alkaline phosphatase enzyme reactivation. In this we have made different concentration of metal solution of 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% by this different concentration we can check the time required for the reactivation of the alkaline phosphatase enzyme in the pasteurized, milk.

We have checked the metal effect on alkaline phosphatase enzyme in pasteurized milk for 24 hours in a water bath (D.K scientific technologies) at 36°C. In this we have checked it by using five different metals one of that is cobalt (Glaxo smithkline pharmaceuticals, 59876209). Here at different time the alkaline phosphatase enzyme gets activate in the pasteurized milk which is shown in the below table,

Table 5. Readings of cobalt test samples

Cobalt solution concentration effect on ALP test						
Time (Hrs)	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
0	Negative	Negative	Negative	Negative	Negative	Negative
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative	Negative	Negative
7	Negative	Negative	Negative	Negative	Negative	Negative
8	Negative	Negative	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative	Negative	Negative
14	Negative	Negative	Negative	Negative	Negative	Negative
15	Positive	Positive	Negative	Negative	Negative	Negative
16	Positive	Positive	Positive	Negative	Negative	Negative



17	Positive	Positive	Positive	Negative	Negative	Negative
18	Positive	Positive	Positive	Negative	Negative	Negative
19	Positive	Positive	Positive	Negative	Negative	Negative
20	Positive	Positive	Positive	Negative	Negative	Negative
21	Positive	Positive	Positive	Negative	Negative	Negative
22	Positive	Positive	Positive	Negative	Negative	Negative
23	Positive	Positive	Positive	Negative	Negative	Negative
24	Positive	Positive	Positive	Negative	Negative	Negative



Figure 21. Readings of cobalt test samples at 0 hour



Figure 22. Readings of cobalt test samples at 15 hour



Figure 23. Readings of cobalt test samples at 16 hour



Figure 24. Readings of cobalt test samples at 17 hour

Calcium (Samir tech chem. Private limited, 910443)

We have checked the activity of metals on alkaline phosphatase enzyme reactivation. In this we have made different concentration of metal solution of 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% by this different concentration we can check the time required for the reactivation of the alkaline phosphatase enzyme in the pasteurized, milk. We have checked the metal effect on alkaline phosphatase enzyme in pasteurized milk for 24 hours in a water bath (D.K scientific technologies) at 36°C. In this we have checked it by using five different metals one of that is calcium (Samir tech chem. Private limited, 910443). Here at different time the alkaline phosphatase enzyme gets activate in the pasteurized milk which is shown in the below table,

Table 6. Readings of calcium test samples

	Calcium solution concentration effect on ALP test					
Time (Hrs)	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
0	Negative	Negative	Negative	Negative	Negative	Negative
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Positive	Negative	Positive	Negative	Negative	Negative
4	Positive	Negative	Positive	Negative	Negative	Negative
5	Positive	Negative	Positive	Negative	Negative	Negative
6	Positive	Negative	Positive	Negative	Negative	Negative
7	Positive	Negative	Positive	Positive	Negative	Positive
8	Positive	Positive	Positive	Positive	Positive	Positive
9	Positive	Positive	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive	Positive	Positive
11	Positive	Positive	Positive	Positive	Positive	Positive
12	Positive	Positive	Positive	Positive	Positive	Positive
13	Positive	Positive	Positive	Positive	Positive	Positive
14	Positive	Positive	Positive	Positive	Positive	Positive
15	Positive	Positive	Positive	Positive	Positive	Positive
16	Positive	Positive	Positive	Positive	Positive	Positive
17	Positive	Positive	Positive	Positive	Positive	Positive
18	Positive	Positive	Positive	Positive	Positive	Positive
19	Positive	Positive	Positive	Positive	Positive	Positive
20	Positive	Positive	Positive	Positive	Positive	Positive
21	Positive	Positive	Positive	Positive	Positive	Positive
22	Positive	Positive	Positive	Positive	Positive	Positive
23	Positive	Positive	Positive	Positive	Positive	Positive
24	Positive	Positive	Positive	Positive	Positive	Positive



Figure 25. Readings of calcium test samples at 0 hour



Figure 26. Readings of calcium test samples at 3 hour



Figure 27. Readings of calcium test samples at 7 hour



Figure 28. Readings of calcium test samples at 8 hour



Figure 29. Readings of calcium test samples at 9 hour

## DISCUSSION

Alkaline phosphatase which is the enzyme present in the milk gets inactivated by undergoing heat treatment at around 72°C for few seconds which coincides with the heat treatment given to the raw milk which is known as pasteurization. Here in raw milk the alkaline phosphatase enzyme is present which can be identified by using the above procedure and as a result it shows yellow colour precipitates because in raw milk Alkaline phosphatase enzyme acts on phosphoester bond of para nitro phenyl phosphate and upon enzymatic hydrolysis of this para nitro phenyl phosphate the para nitro phenol forms as a product and due to this reaction it gives yellow colour. Raw milk will give yellow colour precipitates when we add buffer solution.

While in pasteurized milk it takes time to get reactive again. As shown in result the pasteurized milk will take approximately 6 hour for the reactivation of the alkaline phosphatase enzyme. Here along we have taken the pasteurized milk sample as the Reference sample. As for the five metal samples that are Zinc (S D fine chem. Limited, 20297 k05), Copper (Samir tech chem. Industry, 30310), Cobalt (Glaxo smith Kline pharmaceuticals, 59876209), Calcium (Samir tech chem. Private limited, 910443) and Manganese except for the copper other than that other 4 metal samples of different concentration are showing the reactivation of the alkaline phosphatase enzyme into the pasteurized milk. As shown above in the results the calcium is the first one in which the alkaline phosphatase enzyme gets active and after this in zinc, manganese and cobalt the alkaline phosphatase enzyme gets activated in 0.1% calcium concentration sample. While in copper sample it does not show any activity for 24 hours.

## CONCLUSION

Milk is considered as the whole food because it contains all the essential nutrients and due to this the consumption of milk is higher. To identify the quality of pasteurized milk all dairy industry takes tests in which APL activity test is important to know the inactivation of index microorganism but along with that if we also get to know the presence of metal ions so it will be more helpful to the industry so that instead of two or more test we can identify the presence of ions by APL activity test only.

From above study we can say that the copper metal ion is the highly inhibited one in pasteurized milk while the cobalt is semi inhibiting the reactivation of alkaline phosphatase enzyme. While the calcium is highly exhibiting the reactivation of alkaline phosphatase enzyme along with calcium the cobalt and manganese also exhibits the reactivation of the alkaline phosphatase enzyme.

## FUTURE SCOPE

India is the world's largest milk producer which contributes 22 percent to the global production of milk. So with the highest production it also requires to maintain quality of the milk. This study was aimed to examine the reactivation of alkaline phosphatase enzyme into the pasteurized milk. It is a qualitative test which only shows that the test is negative or positive but further more can be done. In this study we can further go and find the quantity of metal ions present into the milk. Along with this we can also find the individual metal ion effect into the body. We can also further study the influence of different metal ions on the physical properties of pasteurized milk.



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