Electronic Supplementary Material (ESI) for Environmental Science: Nano.

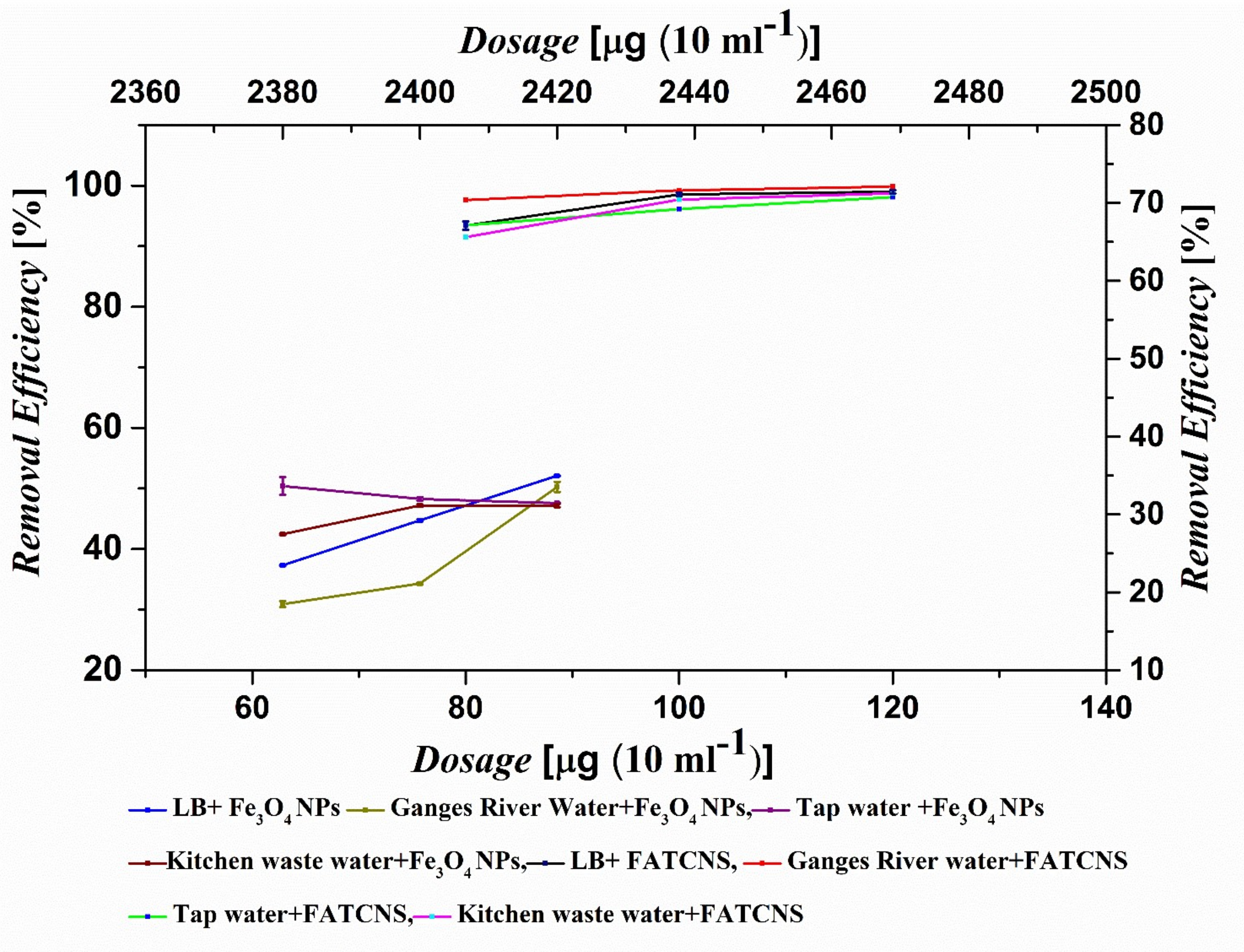
S 3.1. Dosage gradient effect

Results

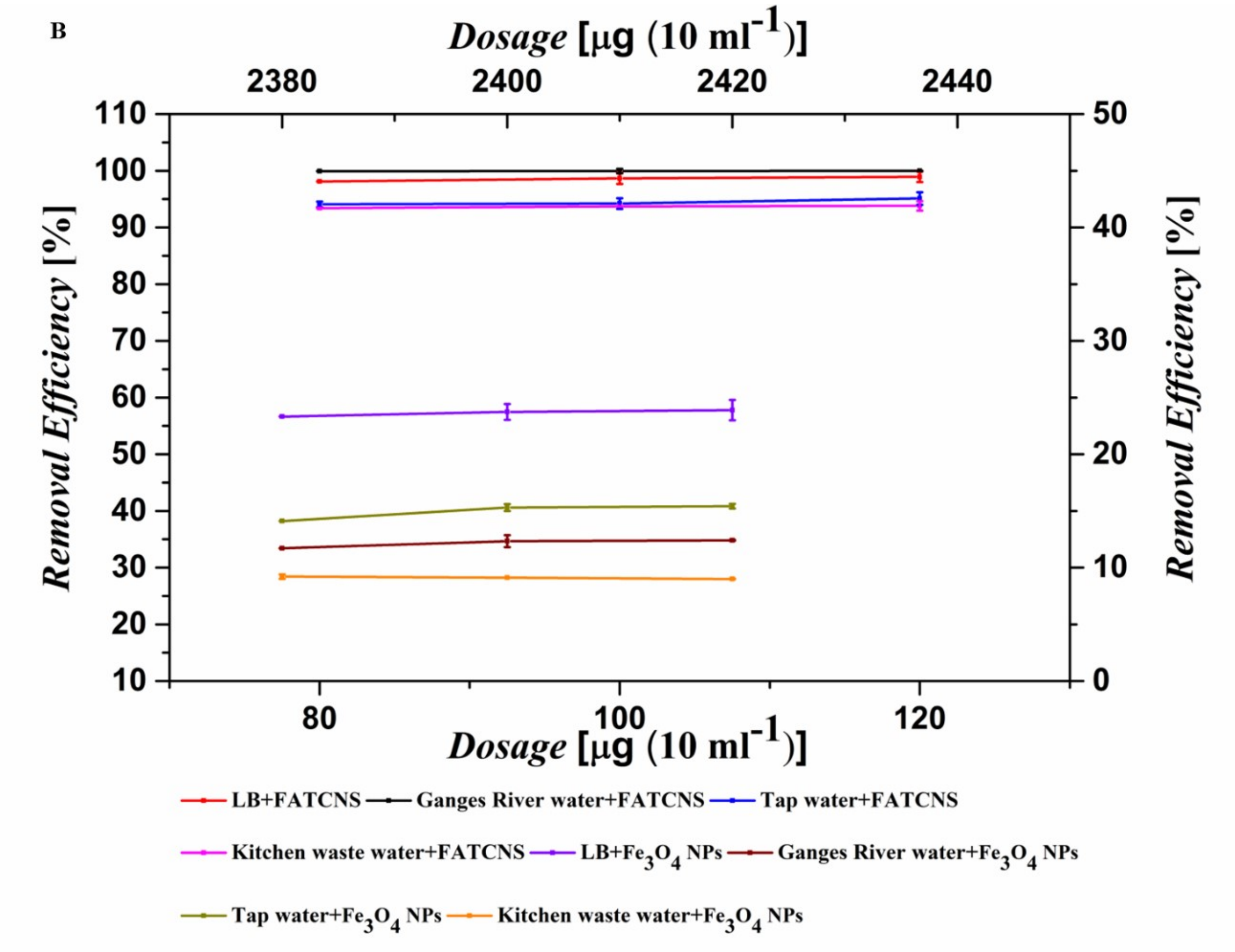
The FATCNS are more efficient in disinfecting water compared to bare Fe_3O_4 NPs. Specifically, only 36 % of *E.coli*, less than 30 % of wtKARBJ1 and less than 20 % of *S.aureus* are removed from LB media using the NPs and their removal efficiencies in river water, tap water and kitchen wastewater are less than 30 % (SI-3 Figure 1). At a dosage of 2420 µg (10 ml^{-1}) NPs consume nearly 90 min to remove all three bacteria from all the water samples. On the other hand, when FATCNS are added in the media with *E.coli* and wtKARBJ1, the natural sediment of the bacterial solution and the absorbance decreased sharply. When maximum dosage of 120 µg (10 ml^{-1}) of FATCNS is utilized, the reaction reaches equilibrium at 1 min, and cell removal efficiencies are greater than 95 % in all the water samples as seen in SI-3 Figures 1 A and B. The NSs show more rapid action than bare NPs and their bacterial removal efficiency, even at extremely low dosage compared to that of NPs, is found to be 63 % higher. Moreover, NPs remain in the treated water after reaction and are difficult to separate. In case of *S.aureus* removal from water (SI-3 Figure 1 C), FATCNS dosage of 60 µg (10 ml^{-1}) in the suspension is more than sufficient to turn the suspension turbidity into precipitate-like aggregation. The precipitate settles at the bottom of the tube within 50 sec.

It is reported that the concentration and the contact time between bacteria and NAs influence the bactericidal and removal efficiency. Further, the cellular uptake of NPs with size greater than 100 nm is low since they are not readily permeable through bacterial cell walls.[1–3] In this study, the adsorption and cellular uptake of NPs may have been low due to their large size (150 nm). Hence, the removal efficiency of NPs is low even at high dosage and long contact time. On the other hand, the desirable size of FATCNS (50 nm) leads to rapid cellular uptake, quick interaction, and adsorption on the bacterial cell wall, which enhances the bacterial removal efficiency and decreases the required time and dosage. Overall, the minimum dosage of 100 µg (10 ml^{-1}) of FATCNS is optimized and further used to evaluate the effect of pH, ionic strength, and co-existing anions on its bacterial removal efficiency.

A



Dosage [μg (10 ml^{-1})]
2360
2380
2400
2420
2440
2460
2480
2500
Removal Efficiency [%]
100
80
60
40
20
80
70
60
50
40
30
20
10
60
80
100
120
140
Dosage [μg (10 ml^{-1})]
LB+ Fe_3O_4NPs
Ganges River Water+Fe_3O_4NPs,
Tap water +Fe_3O_4NPs
Kitchen waste water+Fe_3O_4NPs,
LB+ FATCNS,
Ganges River water+FATCNS
Tap water+FATCNS,
Kitchen waste water+FATCNS



B
Dosage [μg (10 ml-1)]
2380
2400
2420
2440
110
100
90
80
70
60
50
40
30
20
10
Removal Efficiency [%]
50
40
30
20
10
0
Removal Efficiency [%]
80
100
120
Dosage [μg (10 ml-1)]
LB+FATCNS
Ganges River water+FATCNS
Tap water+FATCNS
Kitchen waste water+FATCNS
LB+Fe3O4 NPs
Ganges River water+Fe3O4 NPs
Tap water+Fe3O4 NPs
Kitchen waste water+Fe3O4 NPs

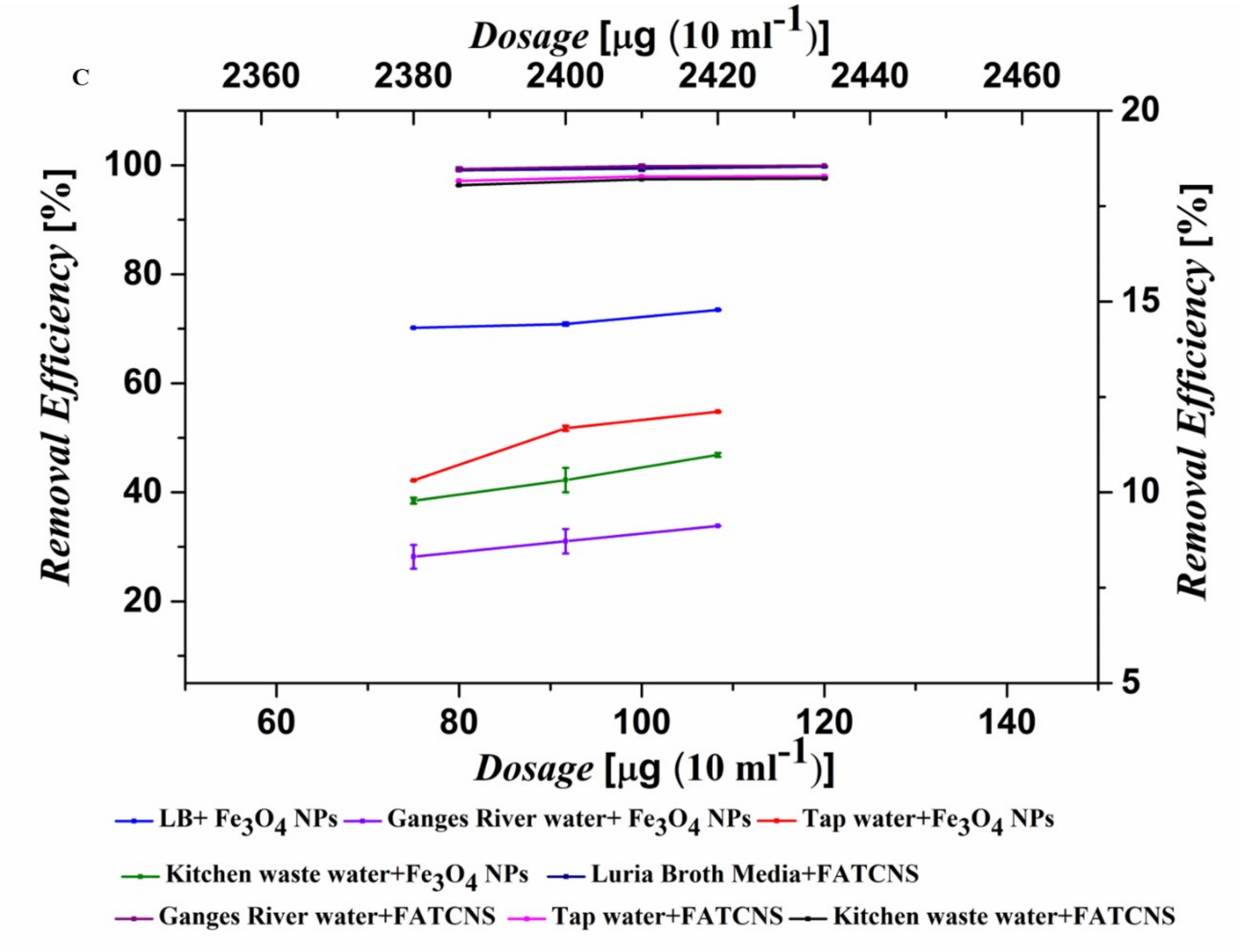


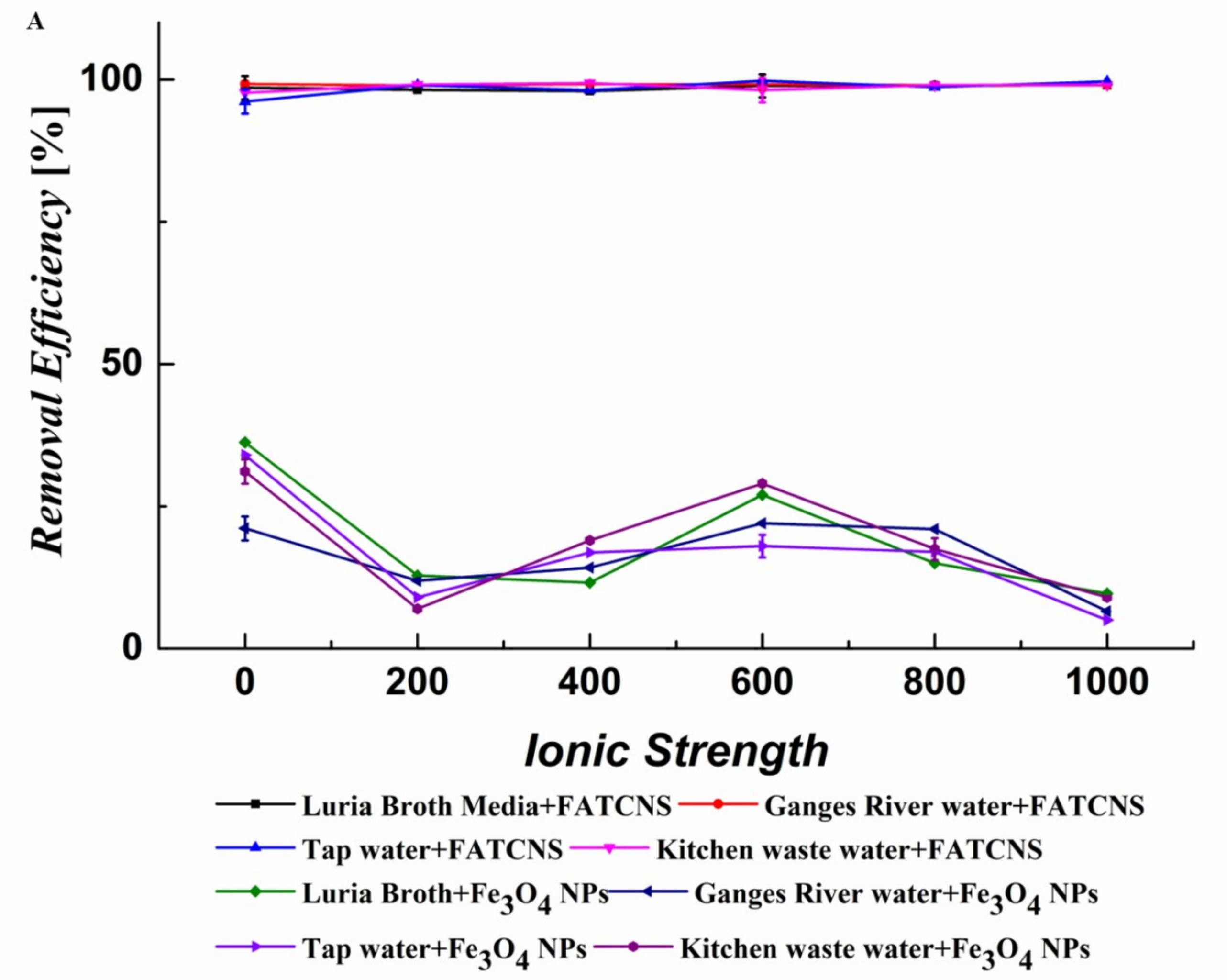
Figure. S 3.1. Bacterial removal efficiency of FATCNS (lower X-axis, left Y-axis) and Fe_3O_4 NPs (upper X-axis, right Y-axis) using their respective MBC for (A) *E.coli*, (B) wtKARBJ1 and (C) *S.aureus*

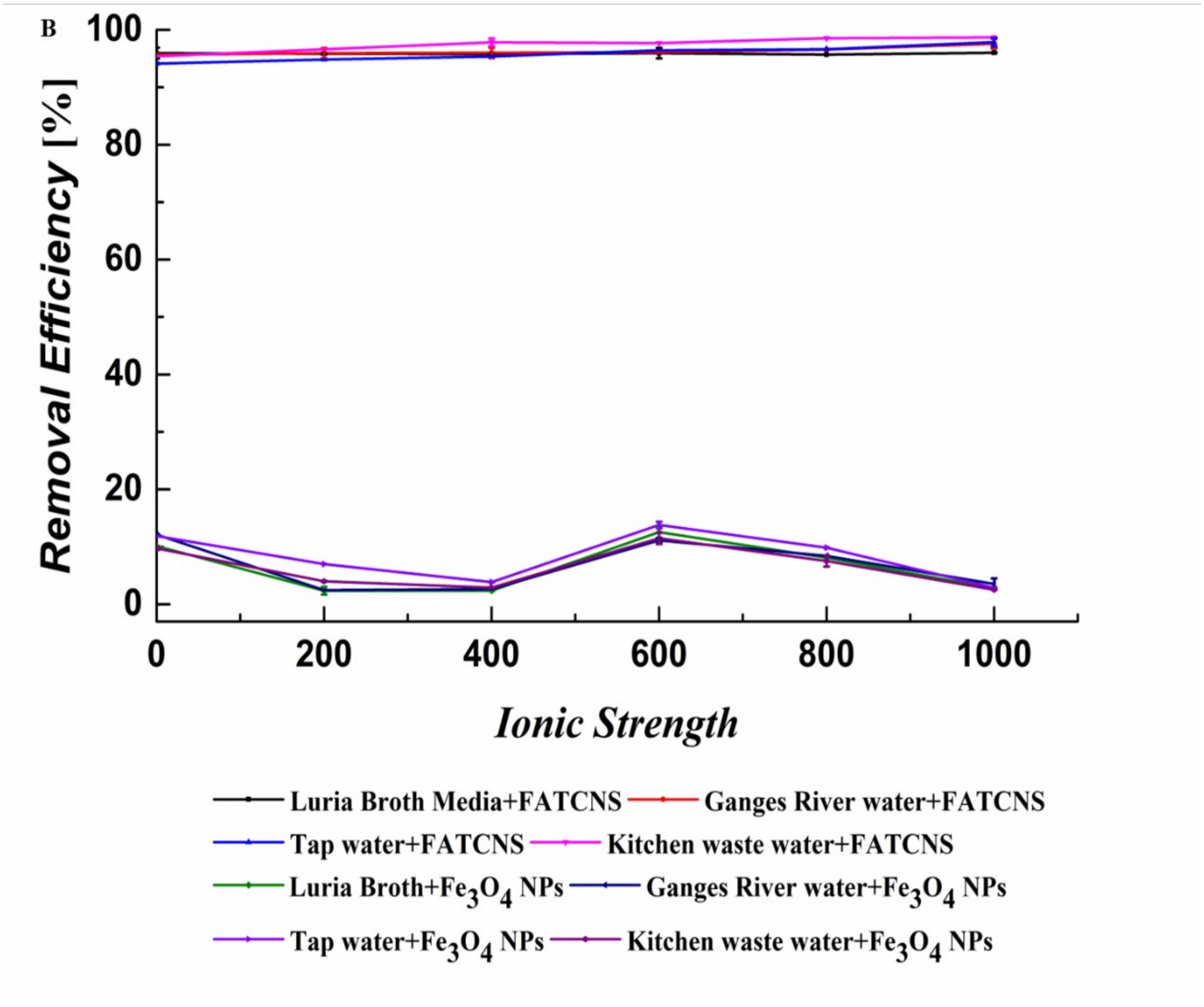
S 3.2. Salinity effect

Results

Similar to pH, the ionic strength of solutions also affects the electrostatic interactions between the bacterial cells and NAs. The effect of the ionic strength on bacterial removal efficiencies is investigated by exposing bacteria (OD_{600}=1.5) to 100 μg (10 ml^{-1}) of FATCNS in a wide range of NaCl concentrations (0-1000 mM) at pH 7.4 for 90 sec. From SI-3 Figure 2, one can observe that FATCNS can effectively capture and remove the bacterial cells over the entire range of NaCl concentrations and for all the tested water samples. The removal efficiency for *E.coli* and *S.aureus* is nearly 100 % in all water samples even at a salt concentration of 1000 mM. The removal capacity for wtKARBJ1 is 96 % in LB, 98 % in river water, 98 % in tap water, and 99

% in kitchen wastewater. The removal efficiency of the bare Fe_3O_4 NPs is seen to be a function of the water sample but attains very low values for all the water samples at the highest NaCl concentrations used. Similar to pH, the positive charge on the surface of the NSs is maintained over a wide range of ionic strength (from +13 mV in 0 mM to + 11 mV in 1000 mM) and leads to an electrostatic attraction and adsorption of NSs on to the cell wall. This adsorption results in complete removal of bacteria even from water solutions with high ionic strength.




B
Removal Efficiency [%]
0
20
40
60
80
100
0
200
400
600
800
1000
Ionic Strength
Luria Broth Media+FATCNS
Ganges River water+FATCNS
Tap water+FATCNS
Kitchen waste water+FATCNS
Luria Broth+Fe_3O_4 NPs
Ganges River water+Fe_3O_4 NPs
Tap water+Fe_3O_4 NPs
Kitchen waste water+Fe_3O_4 NPs

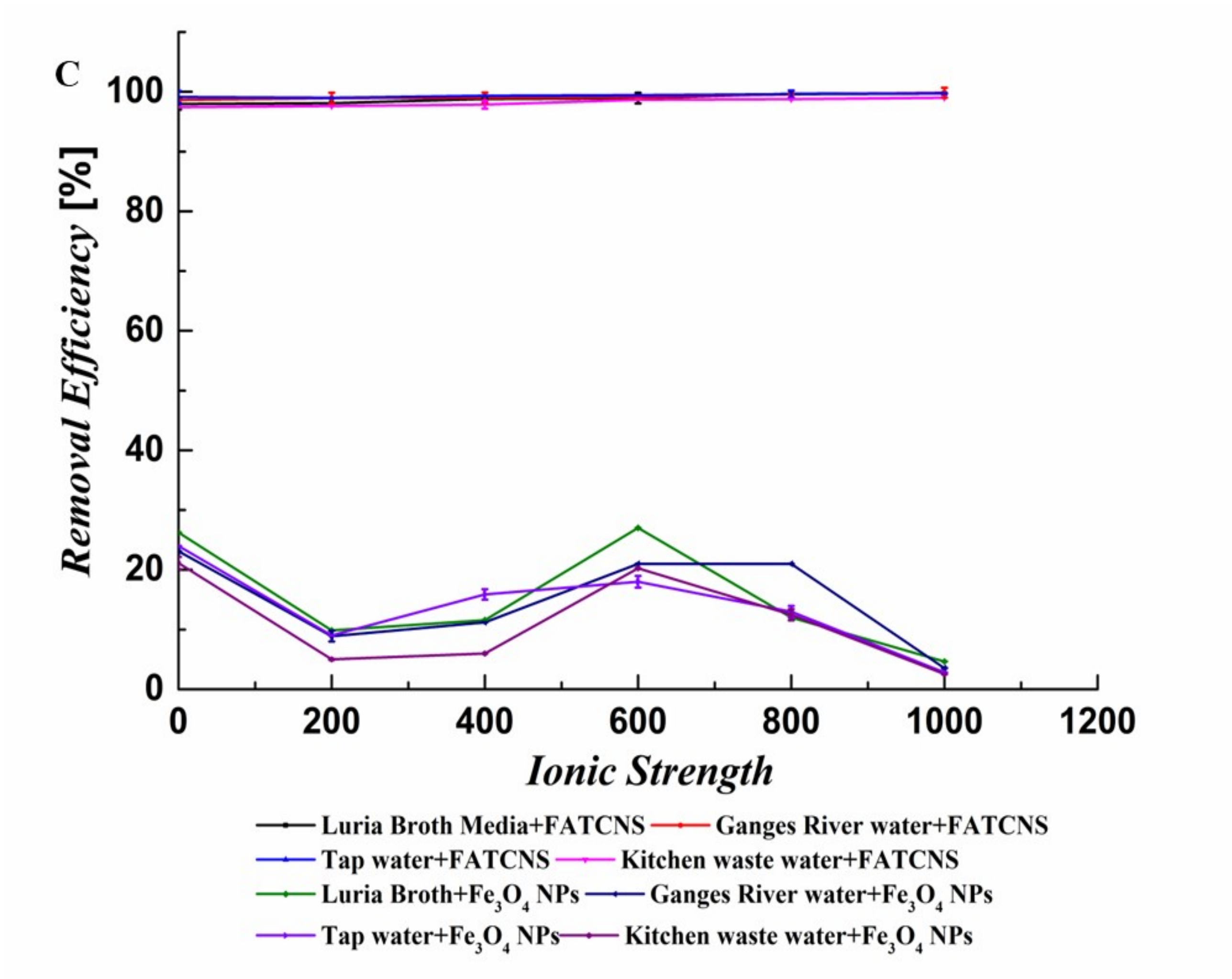


Figure. S 3.2. Effect of the ionic strength on the bacteria removal efficiency of FATCNS (100 µg ($10ml^{-1}$)) and Fe_3O_4 NPs (~2400 µg ml^{-1}) against (A) *E.coli*, (B) wtKARBJ1 and (C) *S.aureus* at pH 7.4.

S 3.3 Co-existing ion Effect

Results

i. This section describes the effects of three anions, viz., nitrates, sulphates, and phosphates, on the bacterial removal efficiencies using Fe_3O_4 NPs. The results are presented in table S 3.3.1. The NPs hardly remove any bacteria from water samples, and the absorbance measured is nearly equal to control suggesting the solid NPs have least interaction with the bacteria in presence of ions in water samples.

ii. Figure S 3.3 presents the bacterial removal efficiency of FATCNS in presence of nitrates+phosphates+sulphates at different concentration in different water samples. The removal efficiency in presence of three ions is greater than 90% suggesting the NSs have the ability to remove the bacteria from different water samples in co-presence phosphates, nitrates, and sulphates.

Co-existing anions	***E.coli***			**wtKARBJ1**			***S.aureus***		
	0.5 mM	**1 mM**	**1.5 mM**	***0.5 mM***	**1 mM**	**1.5 mM**	***0.5 mM***	**1 mM**	**1.5 mM**
Nitrates+LB	25.6	27.1	26.8	5.31	5.11	3.14	8.32	8.15	6.14
Sulphates + LB	24.1	23.5	20.1	4.31	4.15	3.14	10.8	9.65	9.12
Phosphates+ LB	5.21	3.21	2.14	10.2	9.87	7.21	8.15	6.21	4.13
Nitrates+Sulphates+LB	20.4	15.3	11.3	11.3	11	9.21	6	6	5.94
Nitrates+Phosphates+LB	15.8	13.2	10.6	10.5	10.8	9.56	4.3	0.56	0.02
Nitrates+Sulphates+ Phosphates+ LB	11.6	9.00	8.00	11.3	11.5	8.31	10.5	9.21	7.12
Sulphates+Phosphates+ LB	12.3	11.4	9.54	13.4	12.6	8.14	3.15	1.56	0.54
Nitrates + River water	26.1	22.6	18.3	8.21	9.14	8.21	13.5	12.3	9.21
Nitrates+Sulphates+River water	14.6	9.21	2.36	9.21	8.21	6.21	11.5	10.5	9.26
Nitrates+Phosphates+River water	25.3	23.4	8.32	9.47	9	8.51	0.12	0.04	0.04
Sulphates + River water	18.4	14.5	11.84	9	8.14	6.21	10.2	9.54	8.26
Phosphates + River water	3.21	3.14	2.98	8.14	8.14	7.15	9.32	6.32	1.25
Nitrates+Sulphates+ Phosphates+ River water	8.63	8.63	8.1	7.12	9.1	7.58	6.21	4.21	0.89
Sulphates+Phosphates+River water	6.14	6.01	6.00	10.5	11	9.54	5.32	2.36	1.89
Nitrates+Tap water	13.1	12.9	8.32	11.9	11.0	9.21	8.26	8.01	4.21
Sulphates+ Tap water	23.5	20.3	19.9	14.6	13.5	10.6	10.2	9	7.24
Nitrates+Sulphates+Tap water	25.6	22.1	20.1	15.3	16.2	14.6	11.3	9.5	8.15
Nitrates+Phosphates+Tap water	15.6	14.3	11.5	17.5	17.6	12.6	10.6	7.5	6.84
Phosphates+Tap water	13.2	10.6	6.42	18.4	18.2	10.2	5.32	2.3	0.78
Nitrates+Sulphates+ Phosphates+ Tap water	21.1	18.3	13.4	11.6	10.4	9.54	0.74	0.6	0.41
Sulphates+Phosphates+Tap water	23.1	23.2	22.1	15.2	14.6	12.5	0.12	0.1	0.12
Nitrates+Kitchen wastewater	22.1	20.4	20.1	17.4	17.8	12.0	0.16	0.1	0.12
Sulphates+Kitchen wastewater	14.8	14.6	14.2	17.9	17.4	14.6	0.18	0.1	0.1
Nitrates+Sulphates+Kitchen wastewater	9.24	8.21	7.21	18.2	17.6	12.6	0.08	0.08	0.07
Nitrates+Phosphates+Kitchen wastewater	8.21	7.21	2.15	17.2	16.2	10.8	0.08	0.08	0.07
Phosphates+ Kitchen wastewater	3.21	1.26	0.89	16	15.6	9.54	0.01	0.012	0.014
Sulphates+ Phosphates+ Kitchen wastewater	11.0	10.6	9.21	16.8	14.2	8.25	0.02	0.02	0.03
Nitrates+Sulphates+ Phosphates+ Kitchen wastewater	7.06	3.15	0.98	17.6	11.3	6.85	0.01	0.02	0.02

Table S 3.3.1: Effect of co-existing ions on the bacterial removal efficiencies of the Fe_3O_4 NPs against *E.coli*, wtKARBJ1, and *S.aureus*.

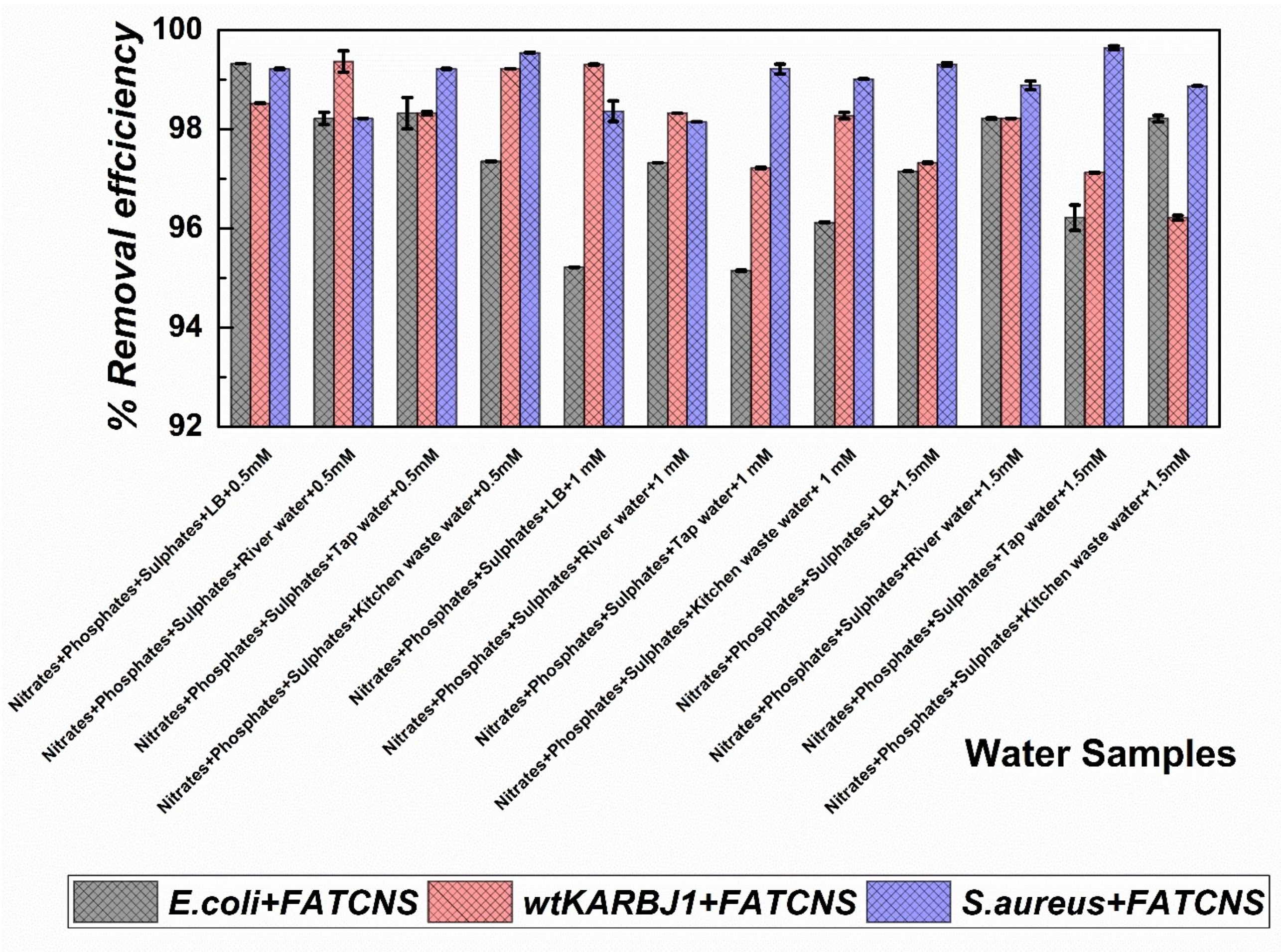


Figure. S 3.3. Effect of nitrates+sulphates+phosphates on the bacterial removal efficiencies of the NSs against *E.coli*, wtKARBJ1, and *S.aureus*.

References

(1) Banerjee, A.; Qi, J.; Gogoi, R.; Wong, J.; Mitragotri, S. Role of Nanoparticle Size, Shape and Surface Chemistry in Oral Drug Delivery. *Journal of Controlled Release* 2016, **238**, 176–185.

(2) Dasgupta, S.; Auth, T.; Gompper, G. Shape and Orientation Matter for the Cellular Uptake of Nonspherical Particles. *Nano Letters* 2014, ***14*** **(2),** 687–693.

(3) Chalkias, N. G.; Kahawong, P.; Giannelis, E. P. Activity Increase of Horseradish Peroxidase in the Presence of Magnetic Particles. *J Am Chem Soc* 2008, ***130*** **(10),** 2910–2911.