International Journal of Environmental Science and Technology
https://doi.org/10.1007/s13762-018-1730-y

ORIGINAL PAPER

# Application of H412R mutant alkaline phosphatase for removal of heavy metals from single-ion solutions and effluents

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Received: 27 July 2017 / Revised: 12 December 2017 / Accepted: 10 May 2018


**Abstract**
Enzyme-mediated bioremediation is an eco-friendly process for removing hazardous toxic heavy metals from the environment. The potential use of mutant alkaline phosphatase H412R for bioprecipitation of heavy metals such as $Co^{2+}$, $Cd^{2+}$, $Cr^{6+}$, $Ni^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ from single-ion solutions and electroplating effluents was analysed in the present study. Purified wild-type and H412R mutant alkaline phosphatase enzymes were incubated with an initial concentration of 100 ppm metal solutions for various time periods along with the substrate *p*-nitrophenol phosphate. Upon catalysis, the enzyme–substrate reaction liberates inorganic phosphate which in turn binds to heavy metals and precipitates them as metal-phosphates. The amount of metal ions precipitated as a result of formation of metal ion-phosphate complexes was determined by estimating the amount of free metal ions present in the solution using atomic absorption spectroscopy. Based on the results obtained, maximum bioprecipitation of metal ions, in general, was observed at 180 min of incubation period. The H412R mutant enzyme exhibited higher efficiency and precipitated 96% of $Mn^{2+}$ from electroplating effluent and 92% of $Co^{2+}$ from the metal ion solution. The pattern of precipitation of various metal ions was in the order $Co^{2+} > Cr^{6+} > Ni^{2+} > Cd^{2+} > Mn^{2+} > Zn^{2+}$ for H412R mutant enzyme and $Co^{2+} > Cr^{6+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Mn^{2+}$ for wild-type enzyme. The results emphasise the use of novel H412R, a mutated alkaline phosphatase enzyme in its catalytic site, as an efficient way of achieving bioremediation of heavy metals from real-time effluents.

**Keywords** Bioprecipitation · *Pseudomonas aeruginosa* · *p*-Nitrophenol phosphate · Toxic heavy metals

## Introduction

Release of hazardous wastes into the environment as a result of tremendous increase in industrialisation and urbanisation is of major public health concern. Contamination of ground water occurs because of discharge of effluents from industries such as tannery and electroplating which are known to contain considerable amount of toxic elements causing toxicity in the ecosystem with consequences to health of humans and animals (Bai et al. 2008; Benazir et al. 2010). Investigations with animal models revealed severe damage to the kidney in mice when exposed to heavy metals at levels beyond the limits set by WHO (Wasana et al. 2017). Among various metals, the trivalent and hexavalent chromium is the metal of concern that has reached a hazardous level of 120 µg/L in the ground water which is alarmingly high (Kazakis et al. 2017). It has been reported that upon continuous exposure chromium could increase the risk of bladder cancer in humans (Wise et al. 2016), while cadmium, even when present in low concentration, accumulates in kidney eventually causing renal failure and cardiovascular diseases (Burke et al. 2016). On the other hand, co-contamination of groundwater with other heavy metals such as iron, cobalt and zinc was found to increase the toxicity of nickel causing severe eczema of skin (Sankhla et al. 2016). Heavy metals are known also to disrupt endocrine system when exposed to prolonged periods of time (Chiu et al. 2016).

Because of long persistence of heavy metals and their associated health hazards several methods have come into existence for the treatment of effluents. Diverse techniques such as physical, chemical, phytoremediation (Huang et al.

Editorial responsibility: Xu Han.

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Published online: 31 May 2018

2017; Sharma et al. 2016; Zheng et al. 2017), bioleaching (Xu et al. 2017) and nanoparticles (Yurekli 2016) were employed during wastewater treatment. Some of these methods involve membrane filtration, ion exchange, adsorption (Zhao et al. 2016; Zhang et al. 2016) and electro-chemical techniques such as electro-winning, electro-dialysis and electro-deionisation (Dermentzis et al. 2011; Xu et al. 2017). Most of these methods are often considered to be expensive, time-consuming and requiring utmost energy during the process. Further, bioremediation processes involving whole organisms face limitations with respect to the availability of metal interactive sites, metal toxicity, biosorption and bioaccumulation. The search for alternate methods which are resourceful, cost-effective, biological and environment-friendly has led to what is known as the field of the green chemistry with emphasis to use of biocatalysts such as enzymes for bioremediation of pollutants (Tischer and Wedekind 1999; Alcalde et al. 2006). Enzymatic bioremediation has become an attractive alternative for the treatment of pollutants since enzymes provide simpler systems than using whole organisms. The added benefit of employing enzymes is that they remain relatively stable during the reactions and being proteins they are readily biodegradable (Ruggaber and Talley 2006).

Several studies have employed commercially available alkaline phosphatases (ALPs; EC 3.1.3.1) to demonstrate the ability of the enzymes for the removal of heavy metals (Chaudhuri et al. 2013a, b). The enzyme, highly conserved from bacteria to mammals, hydrolyses mono- and di-esters of phosphate (Bihani et al. 2011). The crystal structure of *E. coli* ALP was the first to be resolved, and the three-dimensional structure at 1.75 Å revealed that the active site consisted of two $Zn^{2+}$ and one $Mg^{2+}$ cations (Kim and Wyckoff 1991), while the enzyme from *Pseudomonas aeruginosa* was reported to possess higher enzyme activity (Cheng et al. 1970; Ingram et al. 1973). Further, the pho A gene that codes for ALP from *P. aeruginosa* was cloned and expressed in *E. coli* BL21 (DE3) and the enzyme was reported to be 52 kDa in molecular weight (Selvaraj et al. 2016) comprising of 476 amino acid residues (Stover et al. 2000). The contemporary research in bioremediation focuses on the development of innovative techniques for the creation of enzymes with increased selectivity and binding affinity for target metal ions. The mutant ALP H412R from *P. aeruginosa* was shown to possess enhanced enzyme activity with an increase in the turn over number, and it was postulated that the significant increase in $k_{cat}$ in the mutant enzyme might bring out higher precipitation of heavy metals from solutions leading to improved bioremediation capability of the enzyme (Selvaraj et al. 2016).

The present work explores the precipitation of heavy metals using H412R, a novel mutant ALP, from *P. aeruginosa*. The enzyme releases free inorganic phosphate from the substrate, *para*-nitrophenol phosphate (*p*-NPP), which in turn binds to the metal ions and precipitates them as metal-phosphates. Single-ion solutions of heavy metals such as $Cd^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cr^{6+}$, $Mn^{2+}$ and $Zn^{2+}$ were employed for bioremediation studies in addition to electroplating effluent. Precipitation of heavy metals by the wild-type and mutant ALP was analysed using atomic absorption spectroscopy (AAS).

## Materials and methods

### Enzymes and substrate

Wild-type and H412R mutant alkaline phosphatases, purified from *P. aeruginosa*, were used for the precipitation of heavy metals (Selvaraj et al. 2016). After purification the enzymes were stored in aliquots at − 20 °C. The substrate *p*-NPP, obtained from Sigma Chemicals Co., prepared freshly each time was used at a concentration of 2 mM throughout the experiments.

### Buffer

Tris–HCl buffer at a concentration of 0.05 M at pH 8.0 was used during the course of study, and the buffer was stored at 4 °C.

### Metal stock solutions

Stock solutions of metals for $CoCl_2$, $ZnCl_2$, $NiCl_2$, $MnCl_2$, $K_2Cr_2O_7$ and $CdCl_2$ were prepared at a concentration of 1000 ppm and used at a final concentration of 100 ppm in the bioremediation experiments. The salts were dissolved in 1% of $HCl/HNO_3$, and deionised water was used to prepare all the solutions to avoid contamination with metal ions. All the chemicals used in the study were of analytical grade, and the solutions were stored at room temperature.

### Preparation and analysis of electroplating effluents

The effluent was obtained from an electroplating industry situated in Chennai, Tamil Nadu, India. Aliquots of 100 ml of the electroplating effluent were digested overnight, using 5 ml of nitric acid and perchloric acid (5:1), followed by evaporation at 180 °C, reconstitution in 1% of $HCl/HNO_3$ and filtration through Whatman No. 1 filter paper. The metal ions present were quantified by AAS.

### Analysis of heavy metal precipitation

A typical reaction, containing 50 mM Tris–HCl buffer, pH 8.0, 2 mM *p*-NPP and 100 ppm of single-ion solution ($Co^{2+}$,

$Zn^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Cr^{6+}$ and $Cd^{2+}$) along with 1 U of purified enzyme, in a total volume of 2.0 ml, was incubated at 37 °C along with control reactions devoid of the enzyme. All the experiments were carried out in triplicate to minimise sampling errors, and the amount of metal precipitated was monitored at 60, 120, 180 and 300 min of incubation.

The enzymatic reactions were terminated by adding 100 µl of 3 N NaOH after the incubation period, followed by centrifugation at 10,000× *g* for 15 min to pellet the metal-phosphate complexes. The supernatant was subjected to AAS analysis to quantify the free metal ions present in the reactions. The difference between the initial and final concentrations of the metal was considered to be the amount of metal precipitated by the enzymatic reaction during incubation. The amount of metal precipitated from the solutions was derived using the following Eq. 1:

$$x = (a - b)/a \times 100, \quad (1)$$

where $x$ = % of metal precipitation; $a$ = initial conc. of metal in the aliquot; and $b$ = final conc. of metal in the aliquot (i.e. in the supernatant).

## Results and discussion

Ever-increasing industrialisation and awareness on the effects of heavy metals on human health have brought stringency on effluent treatment globally. Now it is feasible to detect metal contamination of food and water relatively easily and accurately due to improvements in analytical tools and methods. The wild-type and mutant forms of ALP were tested and compared for the precipitation of heavy metals in this study, and the enzyme-mediated precipitation follows the biochemical reaction given below:

$$\text{Metal}^{n+} + p\text{-NPP} + \text{ ALP} \rightarrow p\text{-NP} + \text{ Metal}_n(\text{PO}_4)_2 \downarrow$$

### Bioprecipitation of $Co^{2+}$ and $Cd^{2+}$ from single-ion solutions

Triantafyllou et al. (1999) reported the presence of cobalt in the biological food chain which can cause severe health hazards in living organisms. Biosorption of cobalt using biomass from sunflower was conducted by Oguz and Ersoy (2014) under acidic conditions, which is not suitable for treating real-time industrial effluents as they tend to be alkaline. The wild-type and H412R mutant ALPs from *P. aeruginosa* were employed to precipitate cobalt from single-ion solution, and the precipitation obtained is depicted in Fig. 1, and the biochemical reaction follows the equation:

$$Co^{2+} + p\text{-NPP} + \text{ ALP} \rightarrow p\text{-NP} + \text{ Co}_3(\text{PO}_4)_2 \downarrow$$

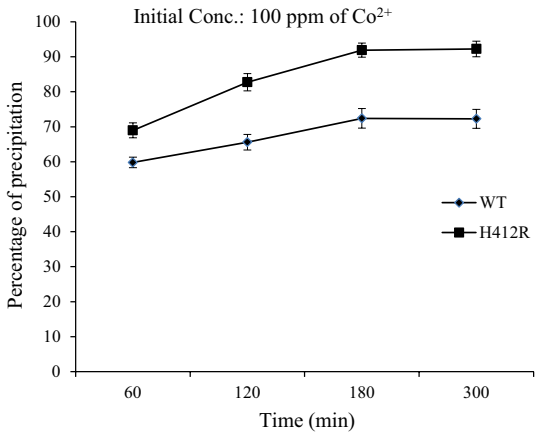


**Fig. 1** Precipitation of $Co^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions. Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (± SEM) of three replications

The mutant ALP, H412R, was earlier reported to have higher catalytic activity and turnover rate in the presence of cobalt (Selvaraj et al. 2016). The improved kinetic parameters have resulted in higher activity in the mutant enzyme which registered 92% precipitation of cobalt, while the wild type could achieve only 72% precipitation during the same incubation period. In a previous study involving *E. coli* ALP inhibition of the enzyme activity by cobalt at pH 8.5 leading to only 20% precipitation of the metal was reported (Chaudhuri et al. 2013b). The observed increase in the enzyme activity with the mutant enzyme, H412R, could be because of rapid release of inorganic phosphate (Pi) from the enzyme which is reflected in terms of threefold higher turnover rate ($k_{cat}$) and sixfold increase in catalytic efficiency. The most striking property observed with the mutant enzyme, H412R, is that in the presence of $Co^{2+}$ the enzyme exhibited an eightfold increase in the enzyme activity compared to the wild-type enzyme (Selvaraj et al. 2016).

As far as cadmium bioprecipitation is concerned the use of several microorganisms was advocated for the purpose (White and Gadd 1996; Bang et al. 2000; Wang et al. 2001). However, specific conditions required for the growth of microbes have been considered as the main disadvantage of this method. Hence, purified enzymes in native state or mutant forms have relevance as they are much more amenable to industrial processes. Bioprecipitation of cadmium using wild-type and H412R mutant ALPs was carried out, and the results are given in Fig. 2. To a larger extent, a linear increase in the percentage of precipitation of cadmium was noticed with the mutant enzyme till 180 min of incubation with a small and steady increase thereafter up to 300 min. In contrast, the wild-type enzyme reached a plateau in terms

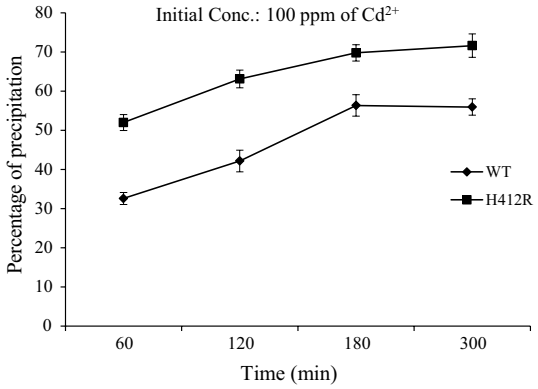


**Fig. 2** Precipitation of $Cd^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions. Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (±SEM) of three replications

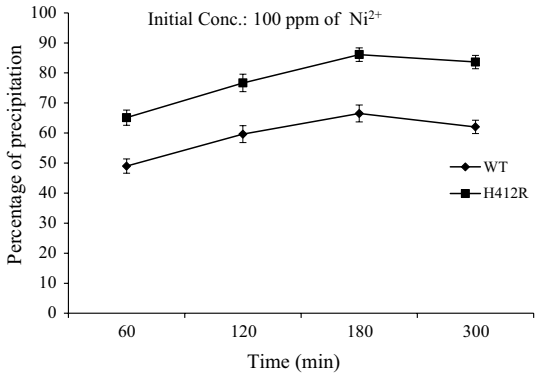


**Fig. 3** Precipitation of $Ni^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions. Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (±SEM) of three replications

of precipitation of the metal ion at 180 min followed by a steady decline subsequently. The mutant enzyme, H412R, resulted in 72% precipitation of cadmium, while the wild-type ALP could achieve only 56% at 300 min of incubation from an initial concentration of 100 ppm of the heavy metal.

The biochemical reaction of cadmium precipitation is as follows:

$$Cd^{2+} + p\text{-NPP} + \text{ ALP} \rightarrow p\text{-NP} + \text{ } Cd_3(PO_4)_2 \downarrow$$

## Bioprecipitation of $Ni^{2+}$ and $Cr^{6+}$ from single-ion solutions

Nickel is one of the major heavy metal contaminants from industries in water and soil. Previously, $Ni^{2+}$ was precipitated as NiS from the wastewater using sulphides. However, the application of the method has been greatly limited by the fact that sulphide itself is a harmful substance to the environment (Pümpel et al. 2003). The precipitation of nickel from single-ion solution achieved by the wild-type and H412R mutant enzymes over an incubation period of 300 min at pH 8 is represented in Fig. 3. The mutant enzyme, H412R, displayed an improved efficiency and precipitated 86% of nickel by 180 min of incubation. This is in contrast to 74% of nickel precipitation by *E. coli* ALP, reported earlier, under similar conditions (Chaudhuri et al. 2013b) and also against only 66% precipitation observed with the wild-type ALP in this study. What is impressive with the mutant enzyme, H412R, is that despite the wild-type enzyme registering less precipitation of nickel compared to the enzyme from *E. coli*, it has surpassed the latter indicating improved architecture of the enzyme due to the site-directed mutagenesis. The biochemical equation of precipitation of nickel is as follows:

$$Ni^{2+} + p\text{-NPP} + \text{ ALP} \rightarrow p\text{-NP} + \text{ } Ni_3(PO_4)_2 \downarrow$$

Unlike the other metal ions, the hexavalent chromium is more toxic to the environment and it is very tricky to remove the metal from the industrial wastes (Krishna and Philip 2005). Recently, enzymes have been found to offer a promising approach to the effective precipitation of $Cr^{3+}$ and $Cr^{6+}$, and ALP was shown to be capable of precipitating the trivalent and hexavalent metal ions, apart from divalent metal ions, through release of Pi from various substrates (Chaudhri et al. 2013b). The results of chromium precipitation achieved with the wild-type enzyme and the mutant enzyme, H412R, are shown in Fig. 4. The mutant enzyme brought out as much as 85% precipitation of $Cr^{6+}$,

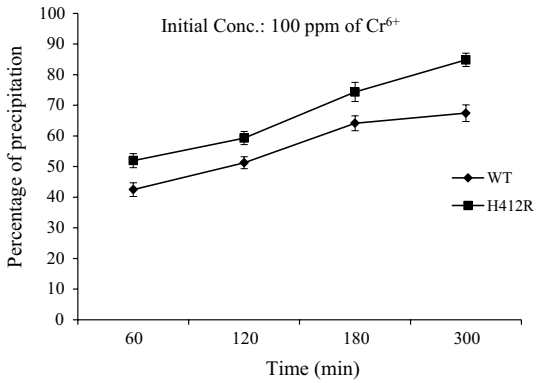


**Fig. 4** Precipitation of $Cr^{6+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions. Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (±SEM) of three replications

during 300-min incubation period, compared to 67% with the wild type and 42% with ALP from *E. coli* reported earlier (Chaudhri et al. 2013b). The results once again affirm the improvement in the catalytic properties in the mutant enzyme. These results demonstrated that the hydrolysis of *p*-NPP and the release of inorganic phosphate were much faster in the H412R mutant compared to the wild-type enzyme. Further, the level of precipitation obtained using the mutant ALP was significantly higher than using the wild-type enzymes from both *E. coli* and *P. aeruginosa*.

The enzymatic reaction leading to precipitation of chromium is as follows:

$$Cr^{6+} + p\text{-NPP} + \text{ALP} \rightarrow p\text{-NP} + Cr(PO_4)_2 \downarrow$$

## Bioprecipitation of $Mn^{2+}$ and $Zn^{2+}$ from single-ion solutions

Manganese is one of the common heavy metal contaminants in industrial effluents. Of late, it has become a major health concern as municipal landfill leachates release it from soil matrix contaminating the ground water reserves (DiPalma and Mecozzi 2010; Abd El-Salam and Abu-Zuid 2015). In the present study, enzyme-mediated precipitation was carried out at 100 ppm of $Mn^{2+}$ single-ion solution at pH 8.0 for various periods of incubation. Unlike with other metals studied, the enzyme from wild type displayed a linear increase in the percentage of manganese precipitated during the entire incubation period of 300 min. The inherent ability of the ALP seems to have been preserved even in the mutant form of the enzyme. As shown in Fig. 5, H412R, mutant ALP, registered 61% precipitation of the metal with a steady increase all throughout, well above and over the levels achieved with wild type, and the latter could catalyse the precipitation to a maximum level of only 41%. However, the overall levels of precipitation obtained with the mutant enzyme were comparatively lesser with manganese compared to other metals used as single-ion solutions.

The overall biochemical reaction for manganese precipitation follows the equation:

$$Mn^{2+} + p\text{-NPP} + \text{ALP} \rightarrow p\text{-NP} + Mn_3(PO_4)_2 \downarrow$$

Various methods have been employed to remove zinc from contaminated water since higher concentration of zinc could lead to various health issues in humans and other animals (Radhika et al. 2006; Lookman et al. 2013). The use of enzymes for the precipitation of heavy metals has drawn attention among researchers since the reaction does not leave any other contaminants in the environment and most importantly the time required for the process to complete is less compared with using whole microbial cells. The purified wild-type and H412R mutant ALPs from *P. aeruginosa* were used for the precipitation of zinc from single-ion solutions. The most prominent observation with the precipitation of $Zn^{2+}$ was that the wild-type enzyme showed higher percentage of precipitation at pH 8.0 in 300 min of incubation compared to the mutant enzyme. Precipitation to the tune of 64% was obtained with the wild type, whereas the mutant enzyme, H412R, resulted in only 55% as shown in Fig. 6. Among all the metal ions precipitated in this study, the effect of zinc on the mutant enzyme was rather inhibitory. Such an effect can be ascribed to the fact that higher concentration of zinc could lead to the reduction in the catalysis reaction which in turn affects the release of Pi from the enzyme (Dean 2002). The biochemical process of zinc precipitation by ALP follows the equation given below:

$$Zn^{2+} + p\text{-NPP} + \text{ALP} \rightarrow p\text{-NP} + Zn_3(PO_4)_2 \downarrow$$

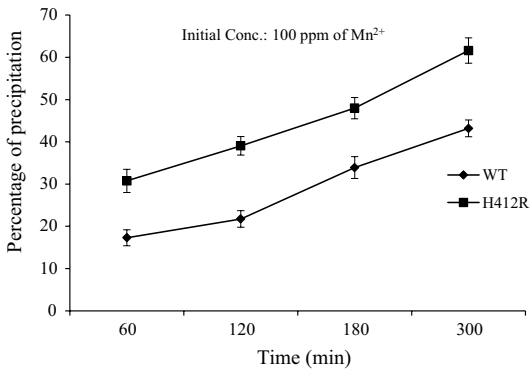


**Fig. 5** Precipitation of $Mn^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (± SEM) of three reactions

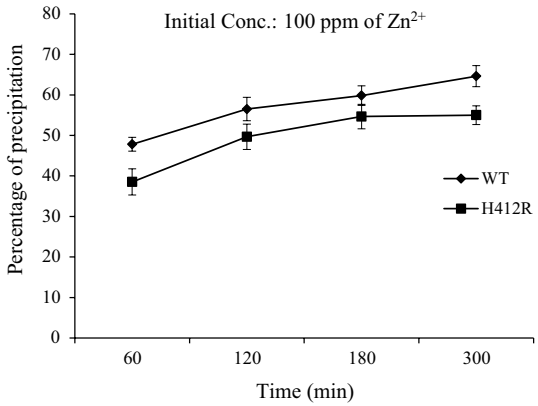


**Fig. 6** Precipitation of $Zn^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from single-ion solutions. Samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C were analysed for metal precipitation. The values correspond to the mean (± SEM) of three reactions

## Pattern of precipitation of metal ions with wild-type and H412R mutant ALPs

A comparison of pattern of precipitation of heavy metals by the wild-type enzyme at pH 8.0 is represented in Fig. 7a. The order of precipitation obtained at 300 min of incubation for 100 ppm metal ion concentration follows: $Co^{2+} > Cr^{6+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Mn^{2+}$. Cobalt resulted in maximum precipitation, whereas the least precipitation was observed with manganese. On the other hand, the precipitation pattern obtained in a previous study using *E. coli* ALP was in the order: $Cd^{2+} > Ni^{2+} > Cr^{6+} > Co^{2+}$ (Chaudhuri et al. 2013b). The results indicate quite opposite affinities for the metal ions by the wild-type ALP from *P. aeruginosa* and *E. coli.* Similarly, the pattern of precipitation of metal ions obtained using H412R, the mutant enzyme, is shown in Fig. 7b and it follows the order: $Co^{2+} > Cr^{6+} > Ni^{2+} > Cd^{2+} > Mn^{2+} > Zn^{2+}$. The variation in the order of precipitation of heavy metals between the wild-type enzymes of the two organisms could be due to the inherent effect of each metal as free radical on the amino acid skeleton of the enzymes. On the other hand, the significant difference obtained in the pattern of precipitation of zinc observed with the mutant ALP might have been as a result of altered catalytic site due to the site-directed mutagenesis which resulted in the replacement of histidine to arginine.

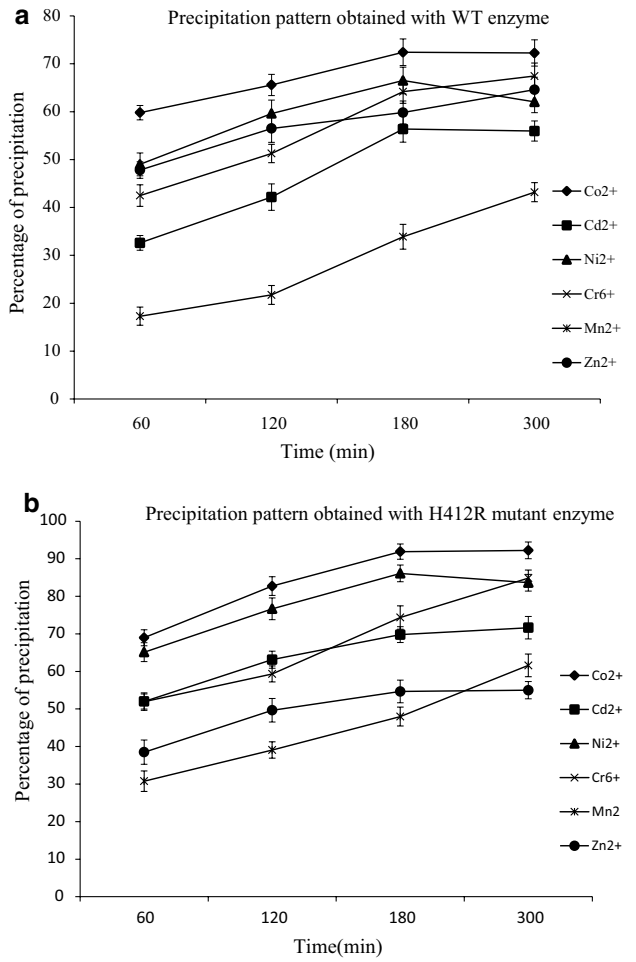


**Fig. 7** **a** Pattern of precipitation of $Co^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Cr^{6+}$, $Mn^{2+}$ and $Zn^{2+}$ obtained from single-ion solutions using wild-type enzyme (WT) at pH 8.0 and 37 °C for various periods of time. The data from Figs. 1, 2, 3, 4, 5 and 6 are pooled and graphically represented. The values correspond to the mean (±SEM) of three reactions. **b** Pattern of precipitation of $Co^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Cr^{6+}$, $Mn^{2+}$ and $Zn^{2+}$ obtained from single-ion solutions using H412R mutant enzyme (WT) at pH 8.0 and 37 °C for various periods of time. The data from Figs. 1, 2, 3, 4, 5 and 6 are pooled and graphically represented. The values correspond to the mean (±SEM) of three reactions

## Bioprecipitation of electroplating effluent

Enzyme-mediated bioprecipitation was validated by applying the technique on the electroplating industrial effluent to precipitate the contaminants, in real time, using the wild-type and the H412R mutant ALP enzymes. The initial concentrations of $Mn^{2+}$ and $Zn^{2+}$ present in the effluent were 34.5 and 3981 ppm, respectively. A maximum precipitation of 96 and 66% for $Mn^{2+}$ was obtained with H412R mutant and wild-type enzymes, respectively, at 300 min after treating the industrial effluent, using *p*-NPP as a substrate (Fig. 8a). The level of precipitation achieved with the effluent from electroplating industry was comparatively higher than with the ones obtained using single-ion solutions. It is significant to note that complete precipitation of manganese was achieved with the mutant enzyme, H412R, under typical reaction conditions.

On the contrary, the precipitation observed for zinc was not much higher with the mutant enzyme compared to the wild type (Fig. 8b) and it was about 53 and 36%, respectively, for the wild-type and mutant enzymes, respectively, at pH 8.0 and 300 min of incubation. Higher concentration of zinc was known to have inhibitory effect on catalysis (Dean 2002; Selvaraj et al. 2016), and the current observation re-establishes the fact as shown in Figs. 6 and 8b with single-ion solution and the effluent from electroplating industry, respectively.

## Conclusion

Of several physicochemical and biotechnological methods available, the use of enzymes immobilised in suitable matrices is gaining importance for bioremediation of heavy metals. The enzyme-mediated removal of toxic heavy metals is more convenient and much reliable since the time period required for the whole process is comparatively less and in addition, no toxic by-products are formed

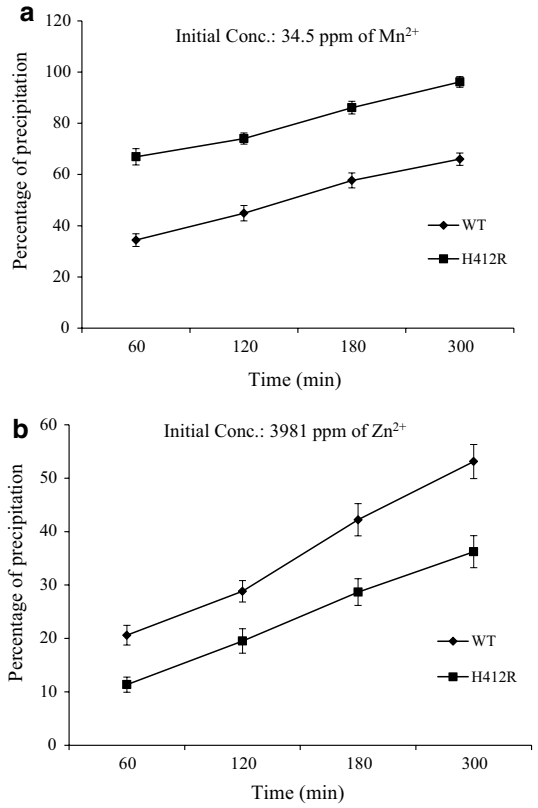


**Fig. 8** **a** Percentage of precipitation of $Mn^{2+}$, using wild-type (WT) and H412R mutant enzymes at pH 8.0, from electroplating effluent. Metal precipitation was analysed in the samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C. The values are the mean (±SEM) of three replications. **b** Percentage of precipitation of $Zn^{2+}$ using wild-type (WT) and H412R mutant enzymes at pH 8.0 from electroplating effluent. Metal precipitation was analysed in the samples withdrawn at 60, 120, 180 and 300 min of incubation at 37 °C. The values are the mean (±SEM) of three replications

during the process. In the present study, ALP from *P. aeruginosa* was explored for the purpose and it was demonstrated that the enzyme subjected to site-directed mutagenesis for replacing histidine with arginine (H412R ALP) had resulted in significant improvement in the enzyme activity with a concomitant increase in the precipitation of various heavy metals such as cobalt, cadmium, chromium, nickel, manganese and zinc from single-ion test solutions as well as manganese and zinc from electroplating effluent. Quite significantly, the mutant enzyme could precipitate almost all manganese present in the electroplating effluent. Thus, it appears that there is ample scope for further research on creating mutations at the active site to achieve a higher turnover number for the enzyme for facilitating more precipitation of metals even with lesser amount of the recombinant enzyme, perhaps in an immobilised state.

**Acknowledgements** We would like to thank SRM University, Tamil Nadu, India, for providing the facilities to perform this research work.

## Abbreviations

ALP: Alkaline phosphatase; AAS: Atomic absorption spectroscopy; H412R: Mutant alkaline phosphatase where histidine is replaced with arginine at 412 amino acid position; $k_{cat}$: Turnover rate; Pi: Inorganic phosphate; *p*-NPP: *para*-Nitrophenol phosphate; ppm: Parts per million

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