Declaration of Authorship

I hereby confirm that I have written the accompanying thesis by myself, without contributions from any sources other than those cited in the text and acknowledgements.

This applies also to all figures, drawings, maps and images included in the thesis.

Freiberg,29.08.2022

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Lalropuia Lalropuia

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ABSTRACT

Indium (In) is one of the most important metals for the modern technology due its use in high tech devices particularly for LCDs and monitors as it exhibits semiconductor and optoelectronic characteristics. It is mainly produced as a by-product of Zinc (Zn) and Zn mainly occurs as sulphides which can be leached by using iron and sulphur oxidising bacteria. Nonetheless, the In recovery could be inhibited by the formation of indium phosphate and indium arsenate, which are poorly soluble. Phosphate (PO_4^{3-}) and Arsenic (As) are usually present in the bioleaching solutions. In this thesis, the effect of PO_4^{3-} and As on the recovery of In during the bioleaching of a sulfidic concentrate (Pöhla concentrate) consisting of mainly sphalerite and a significant amount of arsenopyrite was studied. For this purpose, a series of bioleaching experiments of the Pöhla concentrate using *Sulfobacillus thermosulfidooxidans* were performed: i) using different solid load (SL) 1% ,2.5%, 5% and 10%. ii) using different PO_4^{3-} concentrations- 10%=1.88 mg/L,50%=9.4 mg/L,200%=37.6 mg/L and 500%=94 mg/L of PO_4^3 iii) using different concentrations (60 mg/L or 120 mg/L) of arsenite [As (III)] and (60 mg/L or 120 mg/L) of arsenate [As(V)] for 21 days, at 50^0C. It was found out that 1%SL was the optimum SL where effective recovery of both In (68%) and Zn (80%) was achieved. After ICPMS analysis of the PLS from the experiment with different PO_4^{3-} concentration was done, 200%PO4 has the highest yield of In (81%) and Zn (86%). 500% PO4 has the lowest recovery of In (61%) and Zn (65%). The result of the experiment with different As (III) and As(V) concentration shows that 100% yield of In and Zn were achieved by biotic experiment B(V)60 and chemical control C(III)120. Whereas experiment with added As (III) have low yield of In (34%) and Zn (38%). The intermediate products confirmed by XRD analysis like jarosite, scorodite and elemental sulphur (S) were thought to be the main inhibitory agents for In and Zn recovery.

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List of Abbreviations

C

copper-indium-gallium diselenide **(CIGS),**

D

distilled water **(dH2O)**

E

end of life products **(EOL)**

European Union **(EU)**

I

Indium tin oxide **(ITO)**

Inductively Coupled Plasma Mass Spectroscopy **(ICP-MS)**

L

Liquid Crystal Display **(LCD)**

M

Mackintosh salt **(MAC)**

O

Organic Light emitting Diode **(OLED)**

Oxidation Reduction Potential (ORP)

P

parts per million **(ppm)**

pregnant leach solution **(PLS)**

R

Relative centrifugal force **(rcf)**

revolution per minute **(rpm)**

S

solid load **(SL)**

X

X-Ray Diffraction **(XRD)**

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1.INTRODUCTION

1.1 Indium

Indium [Atomic number 49; Atomic weight=114.82] is a very ductile and malleable post-transition metal belonging to group 13 of the periodic table (Gunn 2014).In is mainly enriched in sulfidic ores like sphalerite and to lesser extent chalcopyrite can also be a carrier as well. The concentration of indium in the earth's crust is about 0.05 – 0.07 parts per million(ppm) (Ulrich Schwarz-Schampera and Peter M. Herzig 2010). In occurs mainly in two oxidation states ,+3(III) and +1(I) and tends to occur with base metals like Cu and Zn .The estimated reserve of Indium is 15,000 tonnes and approximately two third of which is in China (Martin Lokanc et al 2015). Although there are few independent In bearing minerals like roquesite ($CuInS_2$), indite($FeIn_2S_4$) and dzhalindite [$In(OH)_3$] these are not of economic importance. (Cook et al. 2011).

1.2 Economic importance

In has been identified as a critical metal by the European Union (EU) since 2001 (Irrgang et al. 2021).In is generally produced as a by-product of Zn.. (Cook et al. 2011).At present, Indium tin oxide (ITO) which is a transparent conducting oxide constitute the largest use for In. These ITO are used in flat panel display and copper-indium-gallium diselenide (CIGS) is used in solar panels, the demand of which are expected to rise as it has done in the past decade (Moss R et al 2011) .The demand for In is expected to be driven by flat panel display and PV. Also, the speculation in the In market has led to an increase in investment towards the In market. China is the main producer of In. Apart from China, most of these production takes place in Japan, South Korea and Belgium. Since the production of In is concentrated mostly in China and only a few other countries which make the market susceptible to supply restriction or over pricing (Martin Lokanc et al 2015).

1.3 Processing

The most common techniques by which In is extracted is by solvent extraction in Zn refineries. The Zn rich leachate after roasting of Zn ore, which is extracted by conventional mining process, undergoes electrolysis in which Zn is plated onto aluminium cathodes. Around 60% of In is extracted from this Zn leachate by precipitating with jarosite. The remaining insoluble In settles at the bottom of the electrolysis cells along with other trace metals forming Anode Slime. Indium is extracted from the Anode slime residues by dissolving the slime using hydrochloric acid or sulphuric acid and then electrolytic operation is applied for more purification of In (A.M. Alfantazi and R.R. Moskalyk 2003; Gunn 2014).In is also recovered from Cu and Sn concentrates but these are poorly developed. (Gunn 2014)

1.4 Scope for Bioleaching

Since the production of In is dependent on other minerals mainly Zn (to lesser extent Tn or Cu), any change in the production of these minerals would directly affect the In production . The production of In from secondary raw materials could serve as an alternative source which involves the recovery of In from wastes like mine or refinery tailings or from End of Life products (EOL). Bioleaching, a process by which metals dissolution are achieved using microorganisms (Vera et al. 2013), could be the viable option for the production of critical metals like In which are of low grade, and which are produced as a by-product (Rathna and Nakkeeran 2020; Watling et al. 2014). Although bioleaching method of extraction or recovery usually requires longer time compared to other conventional method of mineral processing, its environmental friendliness and cost effectiveness gives them a slight edge (Mishra et al. 2005).

(Jia Feng Li et al.) was able to leached 100% of In from a low-grade Zn ore using *Acidithiobacillus ferrooxidans* .In an experiment by (Martin et al. 2015), nearly 80% of In was recovered from both sphalerite and from Zn-Pb floatation tailings by bioleaching process. There had been a number of studies for the recycling and recovery of In from EOL products using bioleaching. (Pourhossein et al. 2021) from a waste OLED touch screens of mobile phones and (Jowkar et al. 2018) from discarded LCDs both could recover 100% of In using *Acidithiobacillus ferrooxidans.* . Bioleaching could ensure the sustainability and efficiency of the production of such a critical raw material like In. The economic feasibility study for production of critical metals like In from zinc sulphide in Europe and Germany by bioleaching had a positive result. (Irrgang et al. 2021).

1.5 Theoretical background of the thesis

The biooxidation of metal sulphides occurs mainly by the oxidation of Fe and S or both and the ferric and/or proton attack the sulphides resulting in the release of the accompanying metal. (Fowler and Crundwell 1999).The dissolution of metal sulphides could occur in two pathways depending on its acid solubility – Thiosulfate mechanism (Acid insoluble) and Polysulfide mechanism (acid soluble). (Schippers and Sand 1999).The bioleaching of ZnS follows the Polysulfide mechanism.

$$2FeSO_4 + ½\ O_2 + H_2SO_4 \xrightarrow{\text{Bacteria}} Fe_2(SO_4)_3 + H_2O \quad \text{.....(i)}$$

$$S^0 + 3/2\ O_2 + H_2O \xrightarrow{\text{Bacteria}} H_2SO_4 \quad \text{.....(ii)}$$

$$ZnS + ½\ O_2 + H_2SO_4 \rightarrow ZnSO_4 + H_2O + S^0 \quad \text{.....(iii)}$$

$$ZnS + Fe_2(SO_4)_3 \rightarrow ZnSO_4 + 2FeSO_4 + S^0 \quad \text{.....(iv)}$$

Fe (III) oxidises the sphalerite (ZnS) to solubilise Zn^{2+} and form Fe (II) and S^0 (Eqn iv) and the dissolution of ZnS by proton attack results in formation of Zn^{2+} and S^0(Eqn iii). The product of the above dissolution reaction Fe (III) and S^0 can be oxidised by bacteria and hence regenerate Fe(III) and H^+ into the system.(Eqn i &ii).

For this thesis, *Sulfobacillus thermosulfidooxidans.*has been chosen as the bioleaching microorganism which is an acidophilic, mixotrophic and moderately thermophilic bacteria .It is sporulating facultative anaerobe and is both iron and sulphur oxidising bacteria and importantly metal tolerant. (Justice et al. 2014).

Several deposits in Erzgebirge including the Pöhla region are significantly enriched in Indium (Seifert and Sandmann 2006) and efforts have been made for leaching this In rich ore using bioleaching approach. In another separate experiment at the Institute of Environmental Microbiology, TU Bergakademie Freiberg, bioleaching of the sulfidic concentrate or Pöhla concentrate using continuous bioreactor process has been carried out. In this bioreactor experiment, the recovery of In is relatively very low (< 70%) as compared to Zn (≈90%). If the recovery of the In could be improved along with the Zn, it would be of great value.

The most stable oxidation state of In in aqueous solution is In (III) which forms stable chloride and bisulfide complexes. In hydrothermal solutions, the transport of In is contributed by hydroxides, chloride, fluoride or bisulfide complexes but under special conditions sulphate and phosphate may also play a role in the mass transfer of In. At 25^0C, $InPO_4$s are more soluble as compared to In-bisulfide and oxyhydroxides (Wood and Samson 2006). In were able to be precipitated from the pressure oxidative leaching liquor of In containing sphalerite using sodium tripolyphosphate ($Na_5P_3O_{10}$) (Jiang et al. 2011).They found out that, the precipitation

of In increase with increase in pH where 2.6 is the optimum pH constituting 95% precipitation of In. Since the bioleaching experiment is done in acidic condition, this could also be a factor for precipitation of In with PO_4^{3-}. In (III) in aqueous solution has stability limit up to pH 2.5 while at the range of pH 3-5, In_2O_3or $In(OH)_3$ covers the In metal (Fig 1). Since phosphorus (P) is among the basic nutrient requirement for bacteria, the medium in which the experiment is carried out contains phosphate (PO_4^{3-}). The PO_4^{3-} concentration could play a role in the low recovery of In by forming a stable compound with PO_4^{3-} and/or precipitated with it.

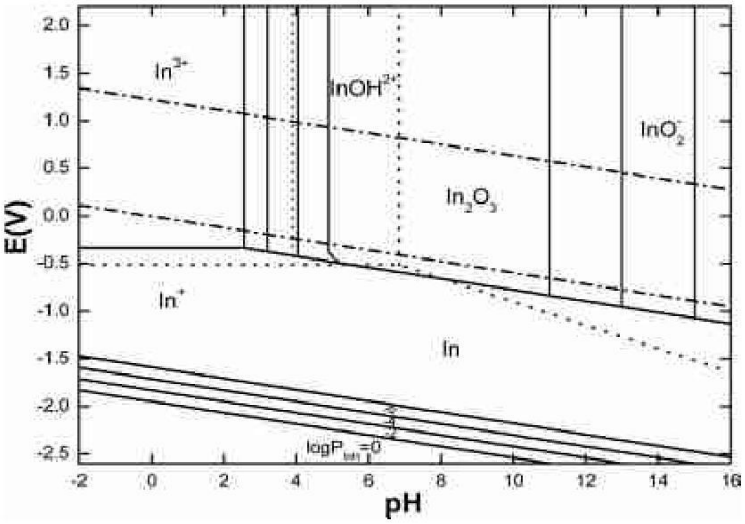


Figure 1: Diagram of ORP-pH equilibrium for In-water system at 25^{0}C (Chung and Lee 2012)

Arsenic (As) usually coexists with metal sulphides and could be toxic for the bioleaching microorganisms and have an inhibitive impact on the bioxidation of Fe or S. (Deng et al. 2020b).The Pöhla concentrate contains (2%) of arsenopyrite(Table 1). The intermediate and secondary minerals formed by dissolution of arsenopyrite like arsenate, arsenite, S^o, jarosite, scorodite and ferric phosphate could contribute to the passivation of the biooxidation (Liu et al. 2019; Corkhill and Vaughan 2009; Yin et al. 2020). According to (Martin et al. 2015) , at pH 1.5-2 , the In precipitated with the As by forming a chemically stable arsenate phase e.g. $InAsO_4.2H_2O$. This was used for processing the pregnant leach solution (PLS) for separation of In from other ions like Zn, Cd, Cu, Al which do not precipitated at low ph. Since the Pöhla concentrate has a relatively high concentration of As (1.2%) and the experiments for this thesis are done at low pH 1.8, there is a high possibility that the In could precipitate with the As and thus influence the recovery of In. To find out whether PO_4^{3-} or As have any effect on the recovery of In during bioleaching of sulphidic concentrate, a series of bioleaching experiments of the Pöhla concentrate were performed using *Sulfobacillus thermosulfidooxidans.*

2.MATERIALS AND METHODS

2.1 Sulfidic concentrate

For the experiments in this thesis, a sulfidic concentrate extracted from Pöhla-Hämmerlein deposit in the Erzgebirge, Germany was used. After extraction, the ore was crushed into 300µm and undergone floatation process to produce the sulfidic concentrate or here forth called as Pöhla concentrate. The elemental composition of the Pöhla concentrate given in g of element/g of the concentrate consists of Fe (11.1%), Cu(0.8%), Zn(18.2%), As(1.2%) and In(0.00018g/1g of ore). Table 1 gives the mineral composition of the Pöhla concentrate after X-Ray Diffraction (XRD) Analysis.

From the bioreactor experiment done previously, it had been observed that the Pöhla concentrate is very alkaline, and the pH rises with time when mixed with Mackintosh salt (MAC) medium. Due to this, the Pöhla concentrate was washed with acetic acid. The quantity of the acetic acid to be used was calculated from the amount of moles of acid that the Pöhla concentrate consumes in the bioreactor experiment (= 0.0009 moles/g of ore). The Pöhla concentrate (1kg) was mixed with (60 ml) glacid acetic acid in a beaker filled with 1L dH_2O and stirred using magnetic stirrer and left it for one night. This concentrate mixture was then washed with distilled water (dH_2O)three times to remove the acid by vacuum filtration (Typ 600p,240mm Rotilabo filter paper) and then dried by leaving it at 60°C oven for two days. The dried Pöhla concentrate were then grinded by hand using Pestle and Mortar and then stored for future use at room temperature.

Table 1 Result of XRD analysis of the Pöhla concentrate

	Sphalerite ZnS	**Quartz** SiO_2	**Pyrite** FeS_2	**Arsenopyrite** FeAsS	**Chalcopyrite** $CuFeS_2$	**Andradite** $Ca_3Fe_2Si_3O_{12}$	**Clinochlor** $(Mg,Fe^{2+},Al)[(OH)_2]Al Si_3O_{10}](Mg,Fe,Al)_3(OH)_6$	**Albite** $NaAlSi_3O_8$	**Hornblende** $Ca_2(Mg,Fe,Al)_5(Al,Si)_8O_{22}(OH)_2$	**Hematite** Fe_2O_3
Volume %	25 ± 2	16 ± 2	1	2 ± 1	2 ± 1	7 ± 1	19 ± 2	12 ± 2	13 ± 2	3 ± 1

2.2 Microorganism and Media

For the bioleaching microorganism type strain *Sulfobacillus thermosulfidooxidans* DSM 9293 was used for all the preceding experiments. Inoculation was done in a sterilized shaking 250 ml flasks using Mackintosh medium (MAC medium) at pH 1.8 consisting of 132 mg/L $(NH_4)_2SO_4$, 53 mg/L $MgCl_2x6H_2O$, 27 mg/L KH_2PO_4 , 147 mg/L $CaCl_2x2H_2O$ and 1 ml of trace elements [76 mg/L $MnCl_2x4H_2O$, 68 mg/L $ZnCl_2$, 64 mg/L $COCl_2x6\ H_2O$, 31 mg/L H_3BO_3,10 mg/L Na_2MoO_4 and 67 mg/L $CuCl_2x\ 2H_2O$]. For source of Fe, 50mM of Fe (II) [$FeSO_4x7H2O$] was used and 0.02% of yeast extract added (both sterilized using filtration). Incubation was done at 50^0C and shaking at 120 rpm.

Fresh cultures of the *Sulfobacillus thermosulfidooxidans* were prepared and grown for all the bioleaching experiments mentioned in this thesis. This was done in order to inoculate the cultures at their exponential growth stage. This freshly prepared cultures (35ml) were centrifuged (Eppendorf Centrifuge 5804R) in two 50ml centrifuge tube at 10000 rcf , for 15 minutes. The supernatant was removed to get rid of the residual ions. This centrifugation step was repeated until the desired number of cells were achieved. This step was done to get the required initial cell concentration which is $1x10^6$ cells/ml without the unwanted ions for all the experiments

2.3 Measurements and preparation

2.3.1 pH and ORP

pH and Oxidation Reduction Potential (ORP) were measured using Mettler Toledo SG2 pH/mV portable meter. The pH/mV meter was regularly calibrated using pH 2 and pH 7 buffer solution.

2.3.2 Ferrozine method

For measuring Fe^{2+}/Fe, a muti-detection microplate reader (SpectraMax M2) using Ferrozine solution was used. Prior to the measurement, each sample is diluted as required using distilled water (dH_2O) at pH 2. First,228µl of Ferrozine solution were added to the Microplate 96 well after which 12µl of each sample (triplicate each) were then added. First measurements were done after 10 seconds shaking and absorption set at 562 nm. After the first measurement, 45µl of hydroxylammoniumchloride solution and 15 µl ammonium acetate buffer were added to the

well. After 20 minutes of incubation at room temperature, the measurement was repeated. Calibration curve was made using 0mM,0.1mM, 0.2mM, 0.4mM, 0.6mM, 0.8mM, 1mM ammonium iron sulphate.

2.3.3 Cell counting

Number of cells were counted using a microscope (Nikon Eclipse E1000) with a counting chamber Neubauer 0.00025 mm^2.Since the solution containing the culture were mixed with Pöhla concentrate, it makes counting of cells very complicated. For this reason, 1:10 dilution was made for the samples to have clearer distinction of the cells from the Pöhla concentrate particles and thus making it easier to count the cells.

2.3.4 ICP-MS

For measuring the concentration of In, Zn, Cu, As, & Fe and for calculation of their respective recovery in the pregnant leached solution (PLS) , Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Thermo Xseries 2) was used. For Samples to be measured, dilution of 1:10000 in 10 ml final volume were made using ultra-pure water 0.055µS/cm in a plastic test tube and 100 µl of 1 µg/L Re, Rh internal standards were added. The tubes were closed with plastic caps and then mixed with vortex.

2.3.5 IC

Ion Chromatography (IC) measurement was done using HPLC Thermo Dionex ICS-5000 DC, 4 mm System. The system was equipped with an ion exclusion column (Phenomenex, Rezex ROA-Organic Acid H^+(8%), 300 x 7.8 mm), using 10mM H_2SO_4 as a mobile phase and with with flowrate of 0.6 ml/min at 50^0C. The injection volume was 25 µl. IC was done to identify the As species [As(III) or As (V)] in the PLS. For this measurement,1:5 dilution was made in 6 ml final volume with 10mM H_2SO_4 in a 6ml vials.

2.3.6 Sterilization

Sterilization was done at 120^{0}C by steam sterilization using VARIOKLAV Dampfsterilisator 400.The Fe (II) [$FeSO_4$x7H2O] solution and the yeast extract were sterilised using filtration (0.2 µm).

2.3.7 XRD-analysis

At the end of each experiment, the solid residue was collected from each flask by filtering using Rotilabo filter paper, Typ 600P, 240mm.The solid residue collected in the filtered paper were then kept at 60^{0}C oven until completely dried. Then, the dried residues are separated from the filter and stored in a reaction tube. The selected samples were sent to the Institute of Material Science of TU Bergakademie Freiberg for XRD-analysis to get the mineral phases of the solid residue.

2.4 Experimental procedures

2.4.1 Bioleaching experiment with different solid load (SL)

To find out the optimum SL of the Pöhla concentrate for the bioleaching experiment, an experiment with different SL(w/v) - 10%,5%,2.5% and 1% was performed in a sterilized 250ml baffled Erlenmeyer shaking flasks with final volume of 100 ml. As mentioned previously (section 2.1), the pH of the media with the concentrate was controlled to pH <1.8 before the freshly grown *Sulfobacillus thermosulfidooxidans* cultures (1x10^6 cells/ml) were inoculated. For each SL, two biotic and one chemical control were prepared. PLS samples were taken every day during the first week and in alternate days from the second week in a 2ml Eppendorf tubes, centrifuged at 20238 rcf for 5 minutes. The supernatant was separated in fresh 2ml Eppendorf tubes and then pH & ORP measurements were taken. The clear supernatant was then stored at 4^{0}C for other measurements. Cells were counted (before centrifugation) on alternative days.

2.4.2 Bioleaching experiment using different phosphate concentration

To find out whether PO_4^{3-} concentration has any effect on recovery of In during bioleaching experiments of the Pöhla concentrate (1%SL), an experiment was performed using different PO_4^{3-} concentrations – 10% ,50%,100%,200% and 500% (corresponding to the PO_4^{3-} concentration present in the MAC medium). Since the MAC medium contains 7.1mg/L of KH_2PO_4(=18.8 mg/L of PO_4^{3-}), 10%=1.88 mg/L,50%=9.4 mg/L,200%=37.6 mg/L and 500%=94 mg/L of PO_4^{3-} For each PO_4^{3-} concentration, triplicates of both biotic experiments inoculated with *Sulfobacillus thermosulfidooxidans* ($1x10^6$ cells/ml) along with their respective chemical control were prepared. The pH of the solution of MAC medium with Pöhla concentrate(1%SL) before inoculation were controlled to pH <1.8 like the previous experiment (section 2.4.1). During the experiment, pH 1.8 was maintained manually by adding 95%H_2SO_4.until the 9th day. Collection and processing of the PLS samples were similar to the previous experiment. (section 2.4.1)

The concentration of In in the Pöhla concentrate is 0.000185 mg/L which is relatively low. 10 times of this i.e 0.00185 mg/L of In (using 1.3g/L of $InCl_3$ solution) were added to all the flasks on the 21st day and the experiment was carried on further for 4 days.

IC measurement was done for PLS samples from the last day of experiment to identify in which species the As were present [As (III) or As(V)]. XRD analysis was done for 200%PO4 biotic and 500%PO4 biotic experiments to find out the difference in the mineral phases formed. ICP-MS measurement was done for selected time points. Other measurements and preparation are similar to the previous experiment. (section 2.4.1)

2.4.3 Bioleaching experiment using different arsenate and arsenite concentration

To find out whether the As has any effect in the bioleaching recovery of In, 1% Pöhla concentrate was bioleached in a sterilized 250 ml shaking flasks using *Sulfobacillus thermosulfidooxidans* ($1x10^6$ cells/ml) inoculated in a MAC medium with different concentrations (60 mg/L or 120 mg/L) of arsenite [As (III)] or arsenate [As(V)]. For As (III) and As (V), 20 g/L $NaAsO_2$ and 10g/L Na_3AsO_4 stock solution were used respectively. Biotic control (where As (III) or As(V) were not added) were also prepared. Triplicates of the biotic experiments [B(III)60 & B(III)120] for experiment with added 60 mg/L&120 mg/L of As (III) and [B(V)60 & B(V)120] with added 60 mg/L & 120 mg/L As(V) respectively were run. Also, chemical control [C(III)120] & [C(V)120] for experiment containing 120 g/L of As (III) and 120 g/L of As(V) respectively were also prepared. During the experiment pH 1.8 was maintained manually by adding 95%H_2SO_4, except between day 10 to 15. Sampling of the PLS was done

similar to the previous experiment (section 2.4.1) at regular interval and pH and ORP were measured for every sample taken.

After the 21st day of the experiment, 120 g/L of As (III) and 120 g/L As (V) were each added separately into the biotic control B1 and B2 respectively. The experiment was further run for 4 days and PLS samples were taken for measurements. For XRD analysis, solid residue from B(III)120, B(V)120 and Biotic control B1&B2 were taken into consideration. Other measurements were similar with the previous experiment (section 2.4.1).

3.RESULTS AND DISCUSSIONS

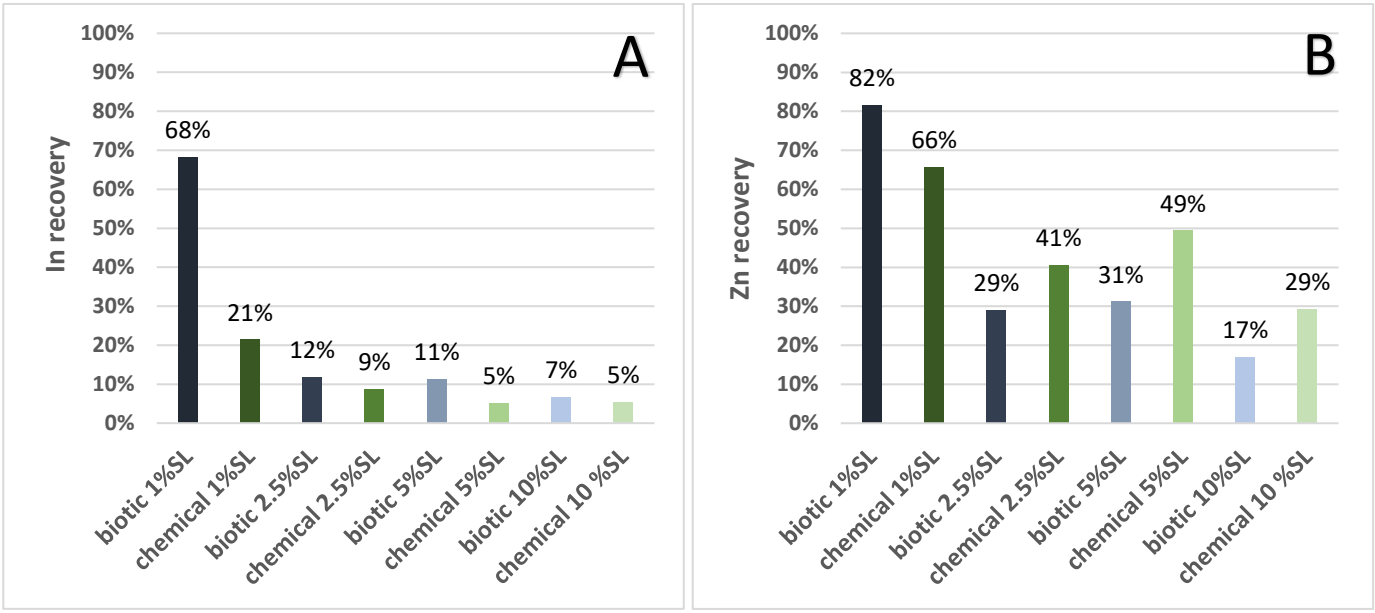
3.1 Bioleaching experiment with different solid load (SL)

To find out the optimum SL for the bioleaching experiment of the Pöhla concentrate, different SL (1%,2.5%,5% and 10%) were leached using *Sulfobacillus thermosulfidooxidans*. After 21 days, the In (68%) and Zn (80%) yield was highest in biotic 1% SL (Fig 1A&B). For biotic experiments >1%SL, the yield of In (<15%) and Zn (< 30%) were very low. Substantial amount of the Zn was leached even in the abiotic experiments and except for 1%SL, the chemical leaching performs better than the biotic ones for Zn (Fig 1B). The pH for biotic 1%SL remains stable around pH 2 after the fourth day while the experiment with higher SLs shows increase in pH with time (Fig 4 A&B). The ORP of biotic 1%SL rises from 360 mV to 515 mV on the fourth day and increase with time up to 555 mV and remain constant till 21st day while the ORP of SLs > 1% remains < 400 and maintain somewhat a straight line (Fig 4 C&D). The cell number in biotic 1% SL is comparatively higher than the experiments with SL>1% (Fig 5). On the same time, the Fe (II) for 1%SL decreases from 45mM on day 3 to 1.8 mM on day 4 and decreases to nearly 0mM from day 12 (Fig 6). This means that the *S.thermosulfidooxidans* was able to completely oxidises the Fe(II) and explains the sudden increase in cell number after day 4 (Fig 5).

For the dissolution of metal sulphides, the oxidative attack is mainly produced by Fe^{3+} and protons. (Schippers and Sand 1999) . The leaching rate of sphalerite decreases greatly at low redox mainly due to the formation of sulphur layers. Therefore, at lower pH (<pH 3) the biological influenced oxidation of Fe^{2+} to Fe^{3+} plays a vital role because at low pH the chemical oxidation of Fe^{2+} is negligible (Vera et al. 2013).In the SL >1%, Fe(II) concentration is more or less constant (Fig 6) indicating that the bacteria were not able to oxidised the Fe(II) and also

the cell numbers were significantly lower than 1%SL (Fig 5).The Pöhla concentrate contains a high amount of silicates like clinochlor, albite and hornblende (Table 1). At low pH, the dissolution of the metals from these silicates results in replacement of the metal with protons. The dissolution of these mineral could therefore increase the pH and even led to jarosite formation. (Dopson et al. 2009).This could explain the increased in pH in SL>1% and inability of the bacteria to strive and oxidises the Fe (II). Therefore, the low recovery of In and Zn for SL>1% could be attributed mainly to the inability of the bacteria to oxidise the Fe (II) to Fe (III) which is the main oxidant for the metal sulphide and the continuous rise of pH due to the dissolution of the silicates.

The experiment with 1%SL has stable pH (<pH 2), have high redox (up to 555mV) and was able to generate Fe (III) by oxidising Fe (II) almost completely. Also, since the pH of SL>1% were never stable, it would be much easier to operate with the 1%SL during the experiment. Therefore, we can conclude that 1% is the optimum SL and hence the preceding experiments have been done with 1% SL.



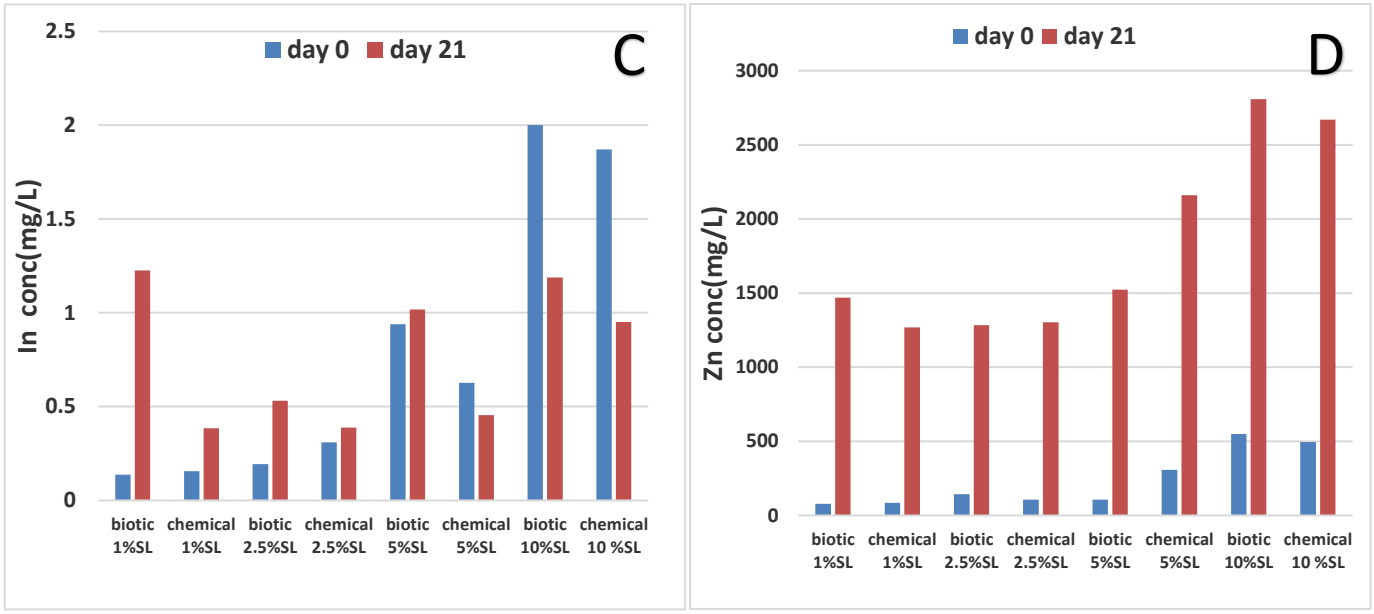
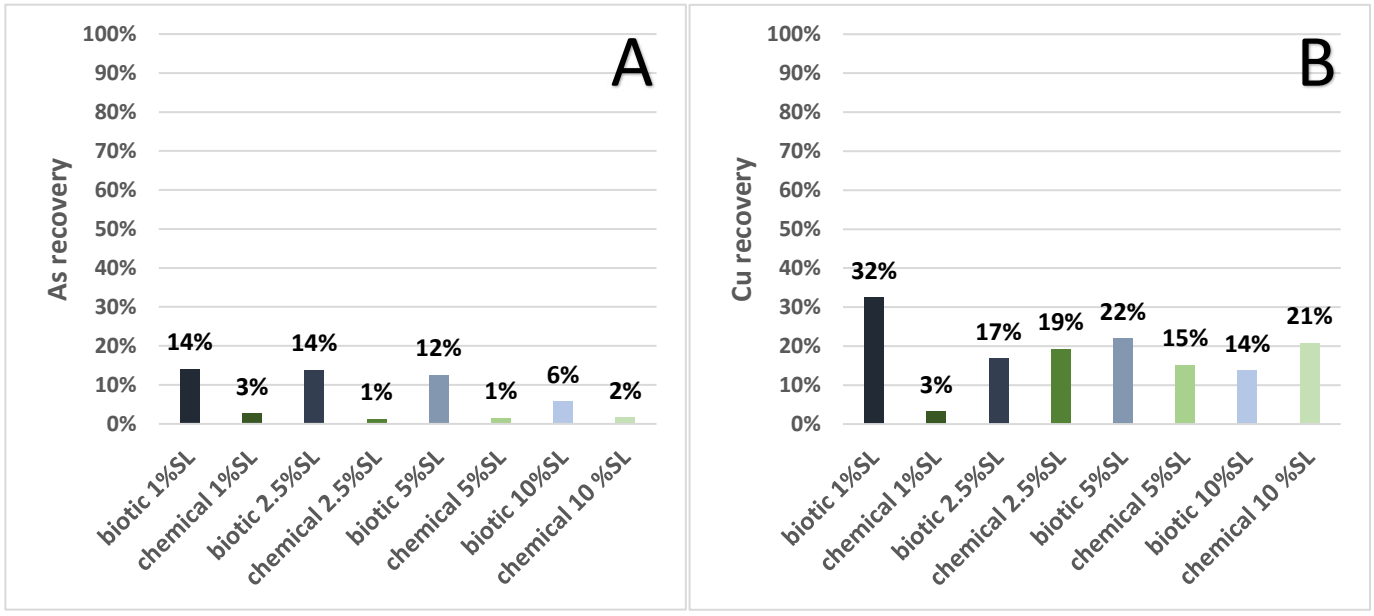


Figure 2:ICPMS measurement for [A&B] the recovery % of In and Zn respectively [C&D] concentration(mg/L) of In and Zn respectively on day 0 and day 21 in the PLS after different SL (1%2.5%,5%,10%) was bioleached using Sulfobacillus thermosulfidoxidans for 21 days



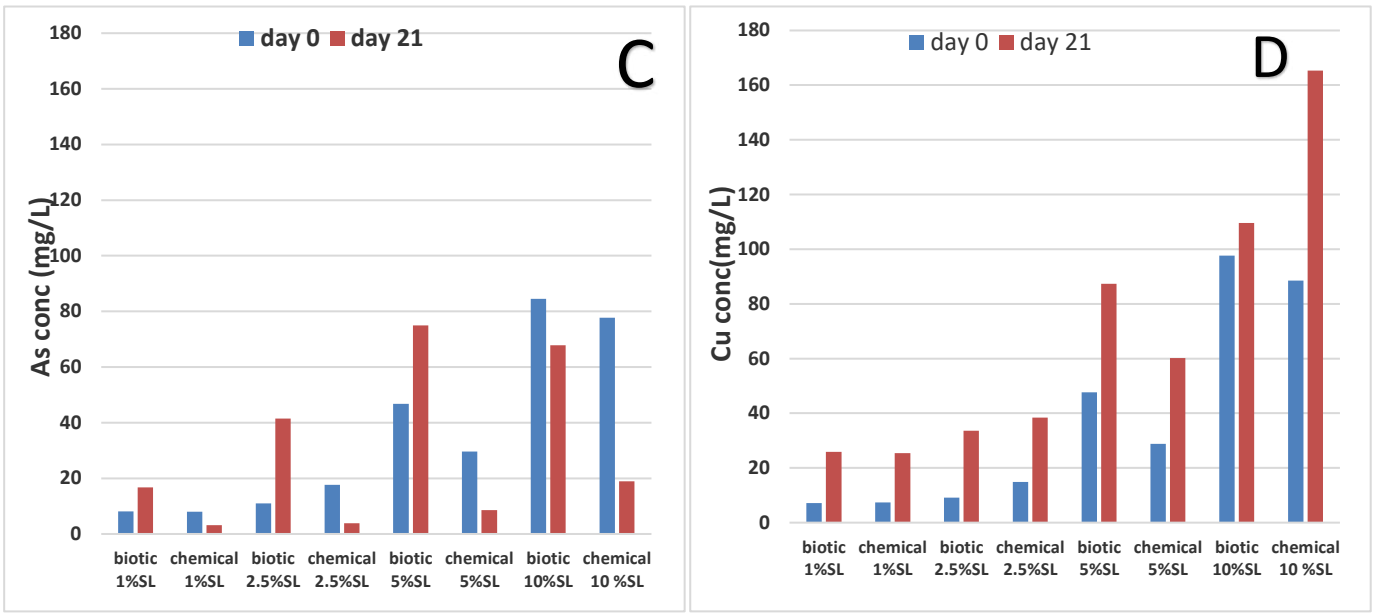
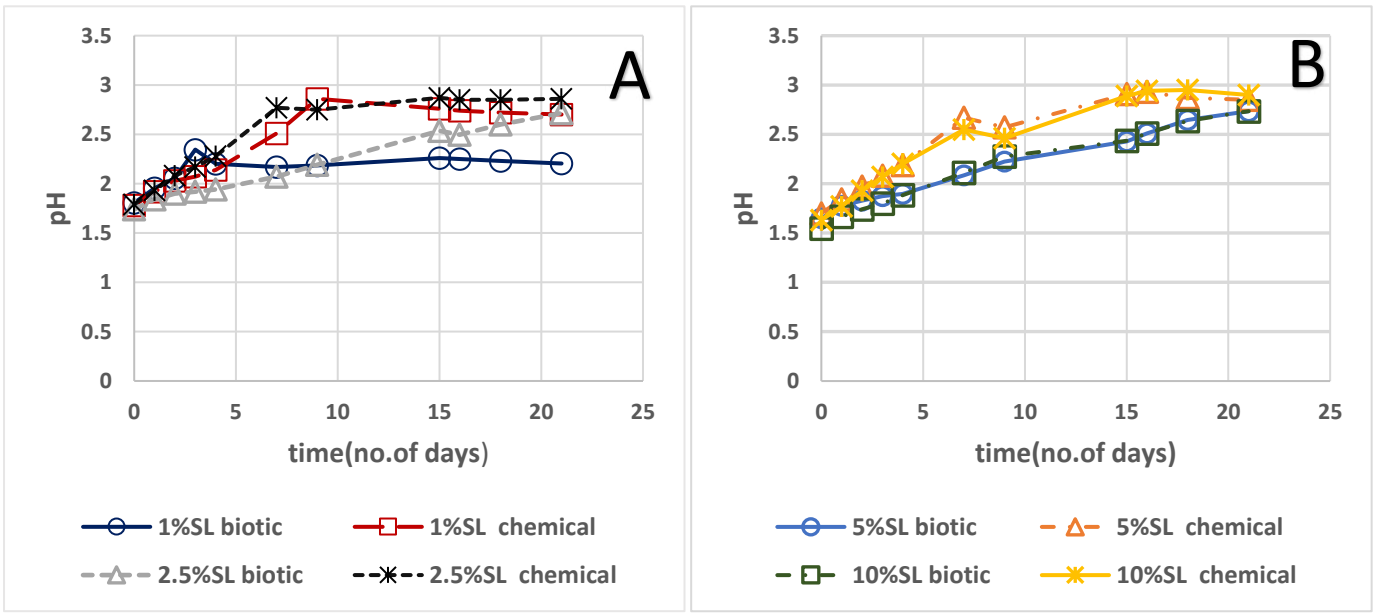


Figure 3: ICPMS measurement [A&B] recovery % of As and Cu respectively [C&D] concentration (mg/L) of As and Cu respectively for day 0 and day 21, after bioleaching experiment of different SL (1%2.5%,5%,10%) of Pöhla concentrate using Sulfobacillus thermosulfidooxidans for 21 days



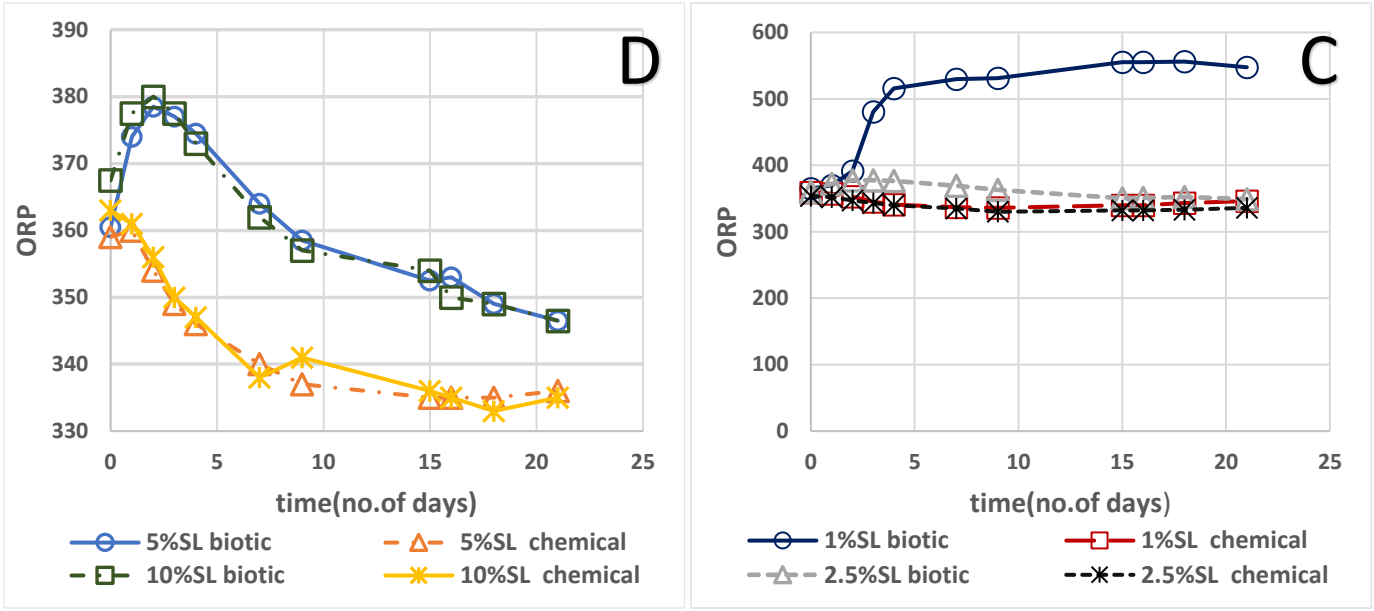


Figure 4: Change in pH [A&B] and ORP [C&D] during the bioleaching experiment of different SL(1%2.5%,5%,10%) of Pöhla concentrate for 21 days using Sulfobacillus thermosulfidooxidans.

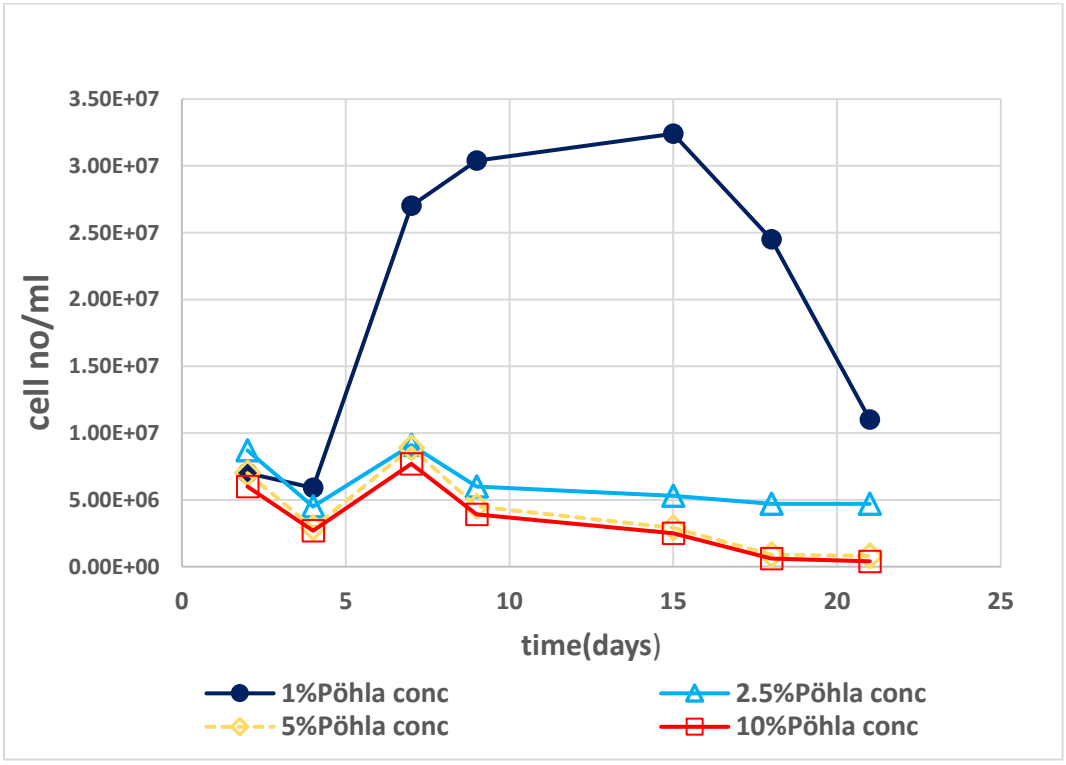


Figure 5:cell counting of Sulfobacillus thermosulfidooxidans during bioleaching experiments of different SL (1%2.5%,5%,10%) of Pöhla concentrate for 21 daysa at 50^{0}C.

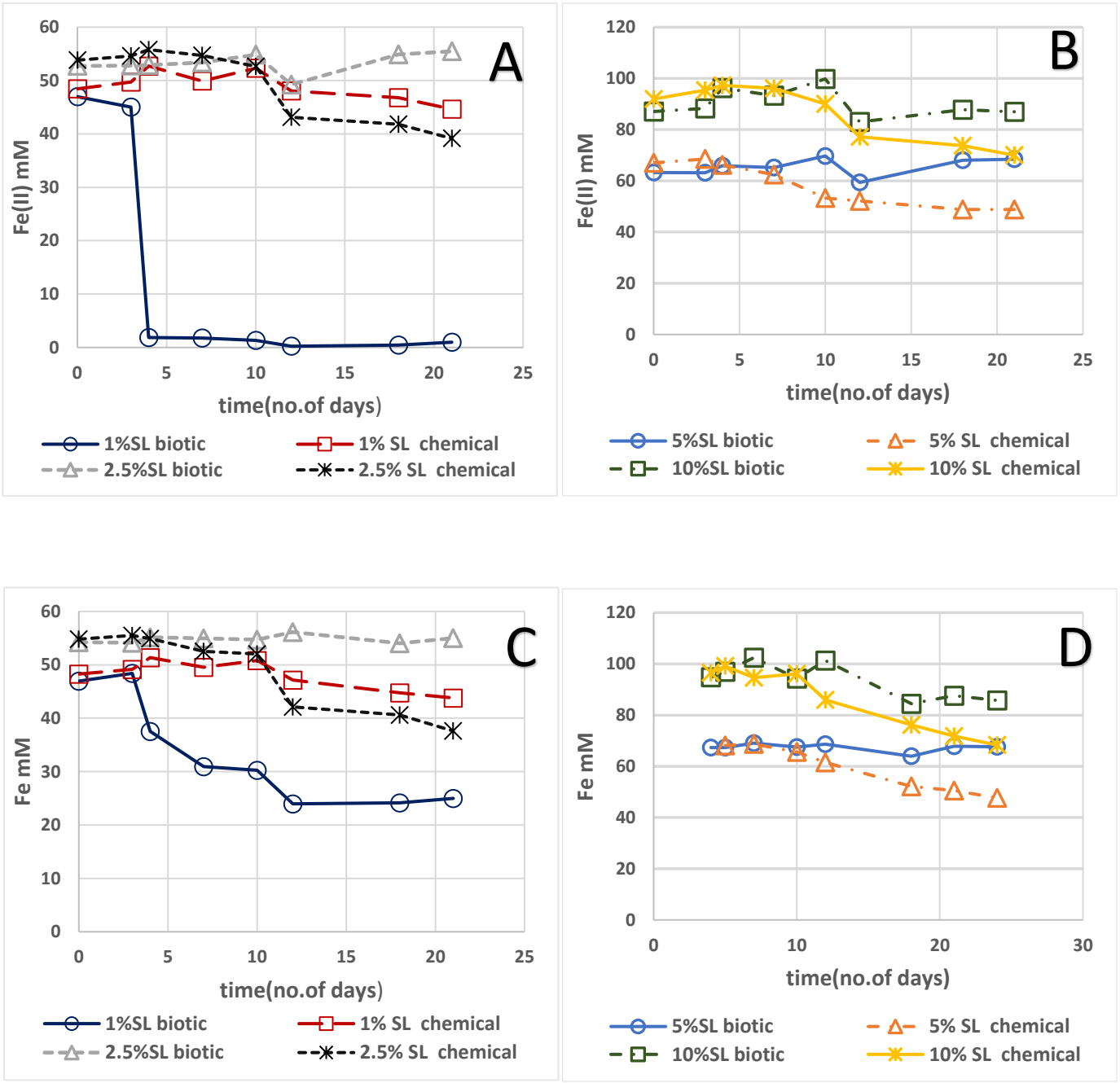


Figure 6: Ferrozine method mesurement of Fe (II) [A&B] and total Fe [C&D] for bioleaching experiment using different SL(1%2.5%,5%,10%) of Pöhla concentrate using Sulfobacillus theromosulfidooxidans.

3.2 Bioleaching experiment using different phosphate concentration

In order to find out the effect of PO_4^{3-} on the recovery of In during the bioleaching of Pöhla concentrate (1%SL), an experiment using different PO_4^{3-} concentrations (10%,50%,100% and 500%) was performed with *Sulfobacillus thermosulfidooxidan*. ICPMS.measurement shows that the concentration of the leached Zn and In in the PLS of both biotic and chemical control is nearly the same till day 10 i.e up until when the pH control H_2SO_4 were used. (Fig 7 A, B;C&D). After that, the concentration in the chemical controls falls behind forming rather a linear rate compared to the biotic ones. This could be the effect of increase in pH (Fig 8 A&B) for the abiotic after day 10 whereas the pH for the biotic remains stable. On the other hand the redox remains within the range of 350-380 mV (Fig 8 C&D) for all the abiotic before and after day 10, which could indicate that the difference in the concentration of In and Zn between the abiotic and biotic after day 10 is solely due to the ph. It has been found that the optimum pH for dissolution of Zn from Zinc sulphide is between pH 1.8-2 and the dissolution decrease as the pH rises(Deveci et al. 2004). At low pH secondary precipitates like jarosite formation is prevented to large extent. Formation of jarosite also depletes the ferric ions which are the main oxidants for sulphide minerals. (Wanjiya et al. 2015) (Liu and Zhou 2022).

For the dissolution of As, there is a clear distinction between biotic and abiotic after day 2 (Fig 7 E&F). At the same time the redox (Fig 8 C&D) for the biotic experiments increase from day 3 while that of the abiotic does not change much. At high redox Fe^{3+} could oxidise As (III) to As (V) . The presence of pyrite or chalcopyrite can also promote this oxidation process. (Deng et al. 2020a). The solubility of As precipitate depends on the Fe/As ratio and the pH. (Krause and Ettel 1989). IC measurement (Fig 9 A&B) shows that in this experiment, almost all the As (III) in the biotic experiments were oxidised to As (V) while in the abiotic ones, the As is in the form of As(III).The total concentration of As (>140 mg/L) (Fig 9 B) in the PLS is higher than the expected total As (=120 mg/L) .This could be explained by the presence of interference in the peak integration report for As(V) produced by the IC indicating that other ion(s) were detected as As(V) by the IC (Fig 9C). The oxidation of As (III) to As(V) is a type of As resistance mechanism for the bacteria (Drewniak and Sklodowska 2013).*Sulfobacillus thermosulfidooxidans* have a high resistance to As (Deng et al. 2020b). The leaching of arsenopyrite is done at high redox. (Ngoma et al. 2018; Deng et al. 2017).Hence, we can see that the recovery of As is higher in biotic experiments (Fig 15C). Unlike the other metals (Zn, In or As), the Cu concentration in the PLS of the abiotic experiments increases with time while that of the biotic ones are lower (Fig 13).This is expected as the leaching of Cu favours low redox for better yield (Lotfalian et al. 2015). After day 10, the Cu concentration in the abiotic elevates as compared to that of the biotic and at the same time the pH also elevates after day

10 in the abiotic. At high pH, the dissolution of the Cu is more dependent on the ORP rather than the ph. (Vilcáez et al. 2008)

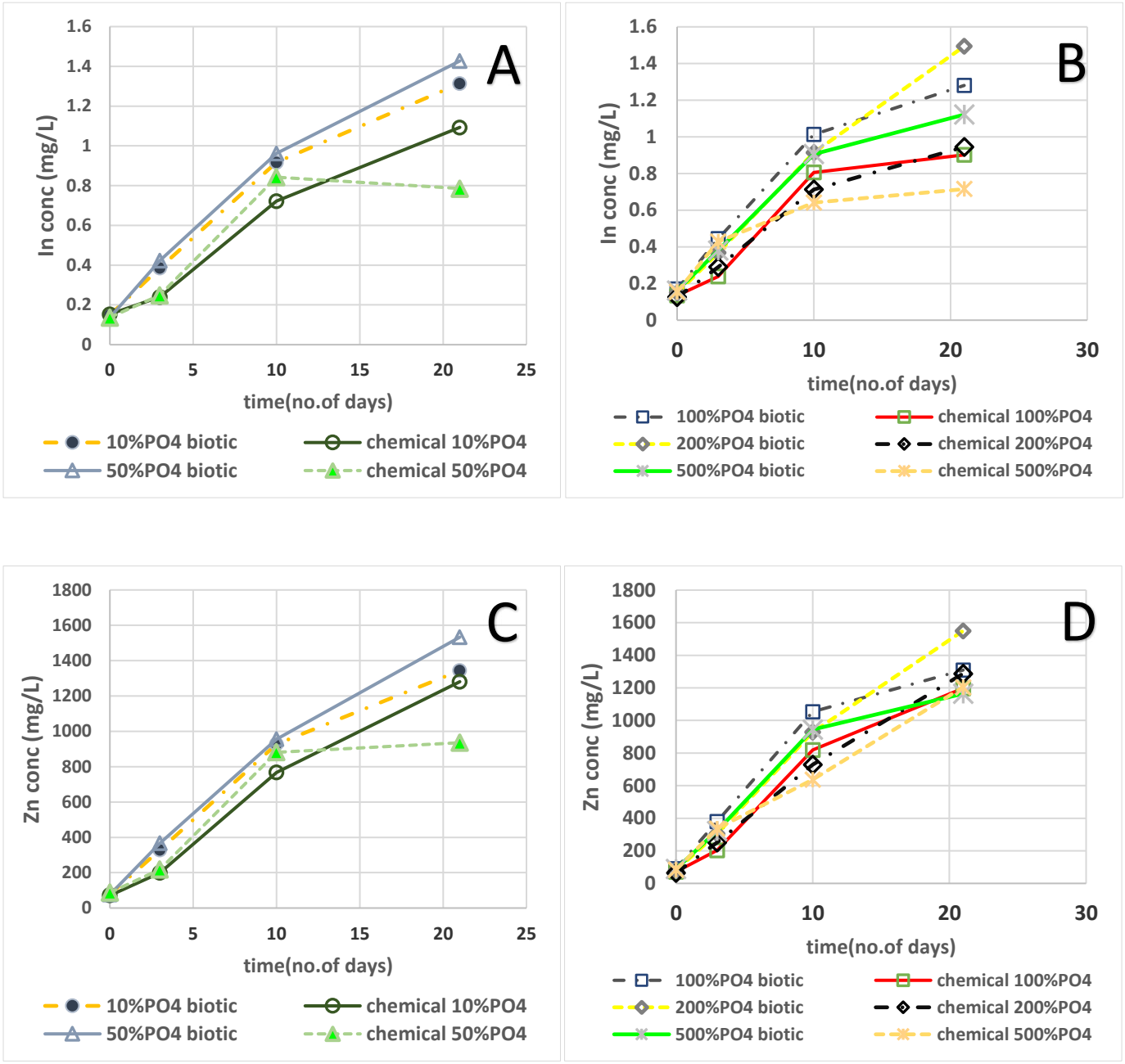
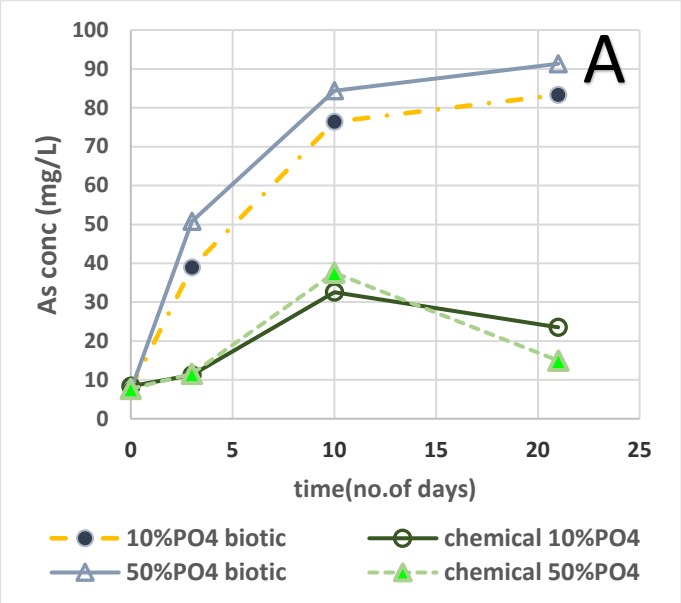
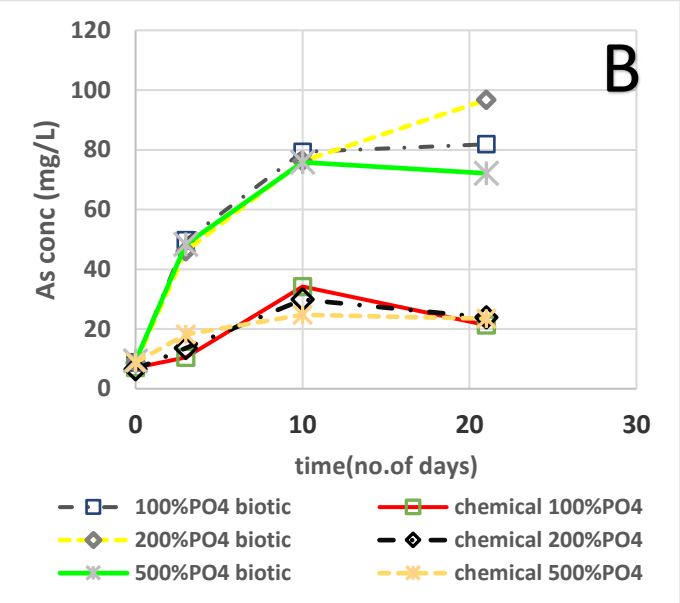
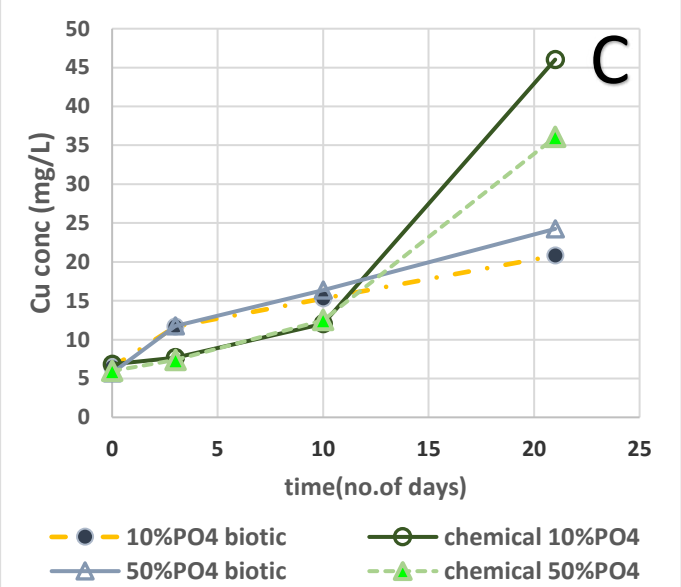
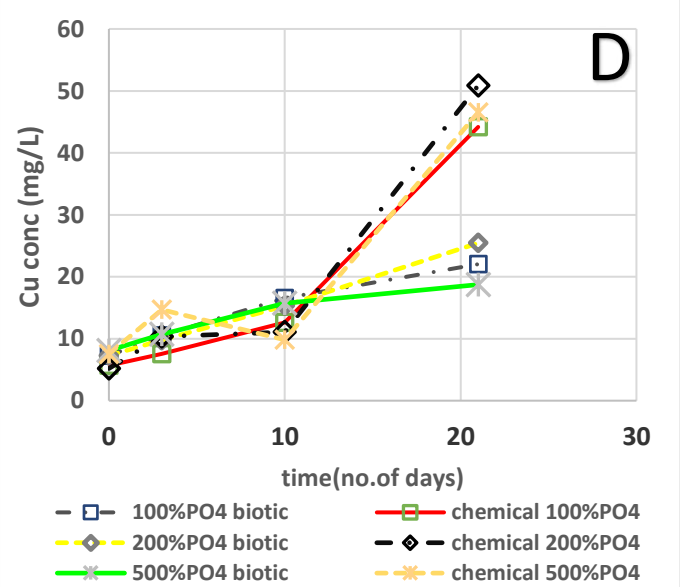


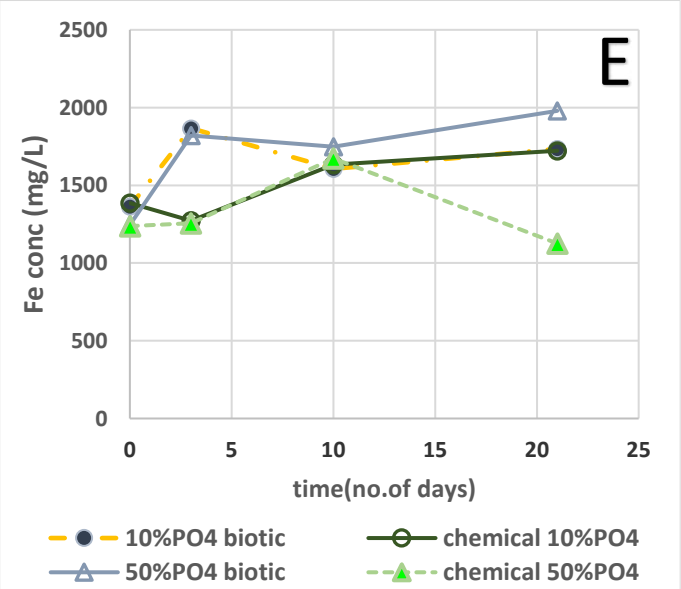
Figure 7:Concentration (mg/L) of In[A&B] and Zn [C&D] after ICPMS measurement for the bioleaching experiment of the Pöhla concentrate (1%) with different PO4 concentrations (10%,50%,100%,200% and 500%).


A
As conc (mg/L)
time(no.of days)
10%PO4 biotic
50%PO4 biotic
chemical 10%PO4
chemical 50%PO4


B
As conc (mg/L)
time(no.of days)
100%PO4 biotic
200%PO4 biotic
500%PO4 biotic
chemical 100%PO4
chemical 200%PO4
chemical 500%PO4


C
Cu conc (mg/L)
time(no.of days)
10%PO4 biotic
50%PO4 biotic
chemical 10%PO4
chemical 50%PO4


D
Cu conc (mg/L)
time(no.of days)
100%PO4 biotic
200%PO4 biotic
500%PO4 biotic
chemical 100%PO4
chemical 200%PO4
chemical 500%PO4


E
Fe conc (mg/L)
time(no.of days)
10%PO4 biotic
50%PO4 biotic
chemical 10%PO4
chemical 50%PO4

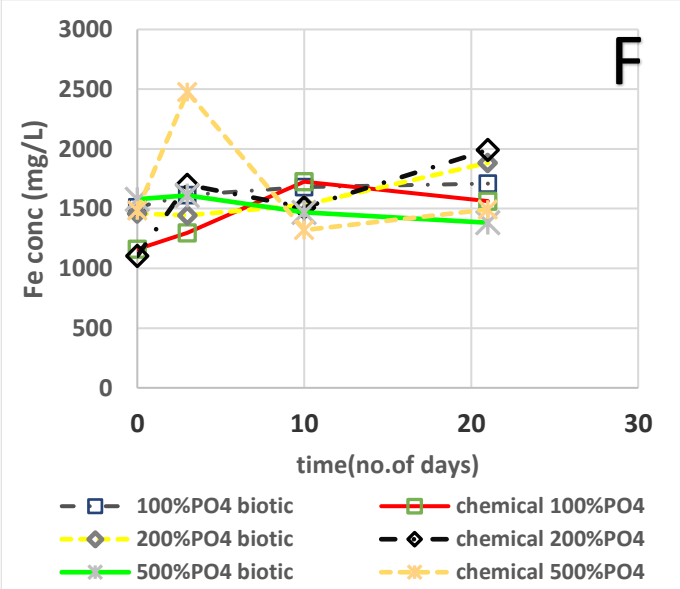

F
Fe conc (mg/L)
time(no.of days)
100%PO4 biotic
200%PO4 biotic
500%PO4 biotic
chemical 100%PO4
chemical 200%PO4
chemical 500%PO4

Figure 8: Concentration (mg/L) of As[A&B],Cu[C&D] and Fe[E&F] after ICPMS measurement for the bioleaching experiment of the Pöhla concentrate (1%) with different PO4 concentrations (10%,50%,100%,200% and 500%).

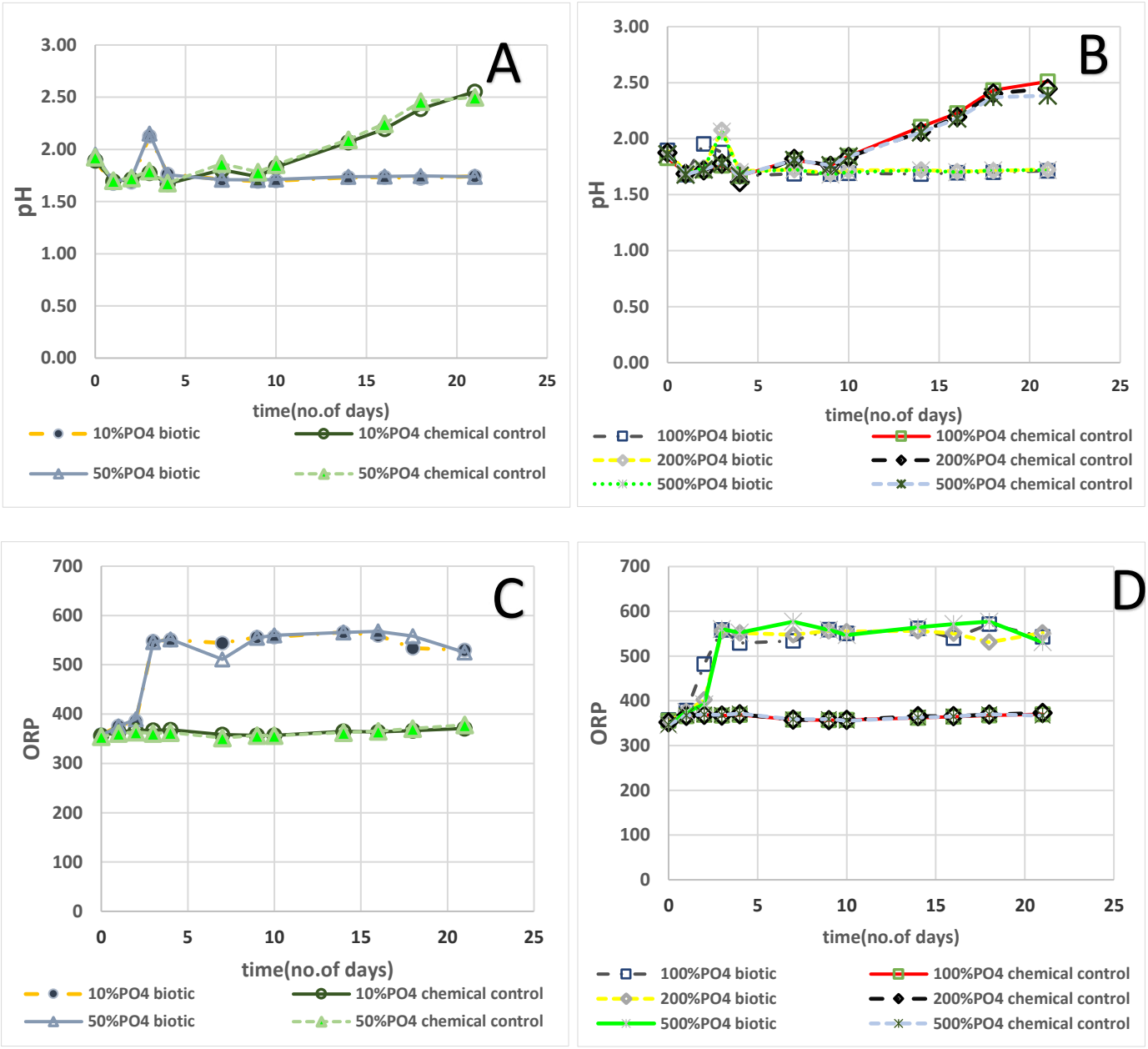


Figure 9: Change in pH [A&B] and ORP [C&D] during the bioleaching experiment of Pöhla concentrate (1%) using different PO4 concentration (10%,50%,100%,200% and 500%) for 21 days where 1.8 pH was controlled using 95% H_2SO_4 till the 9^{th} day of the experiment.

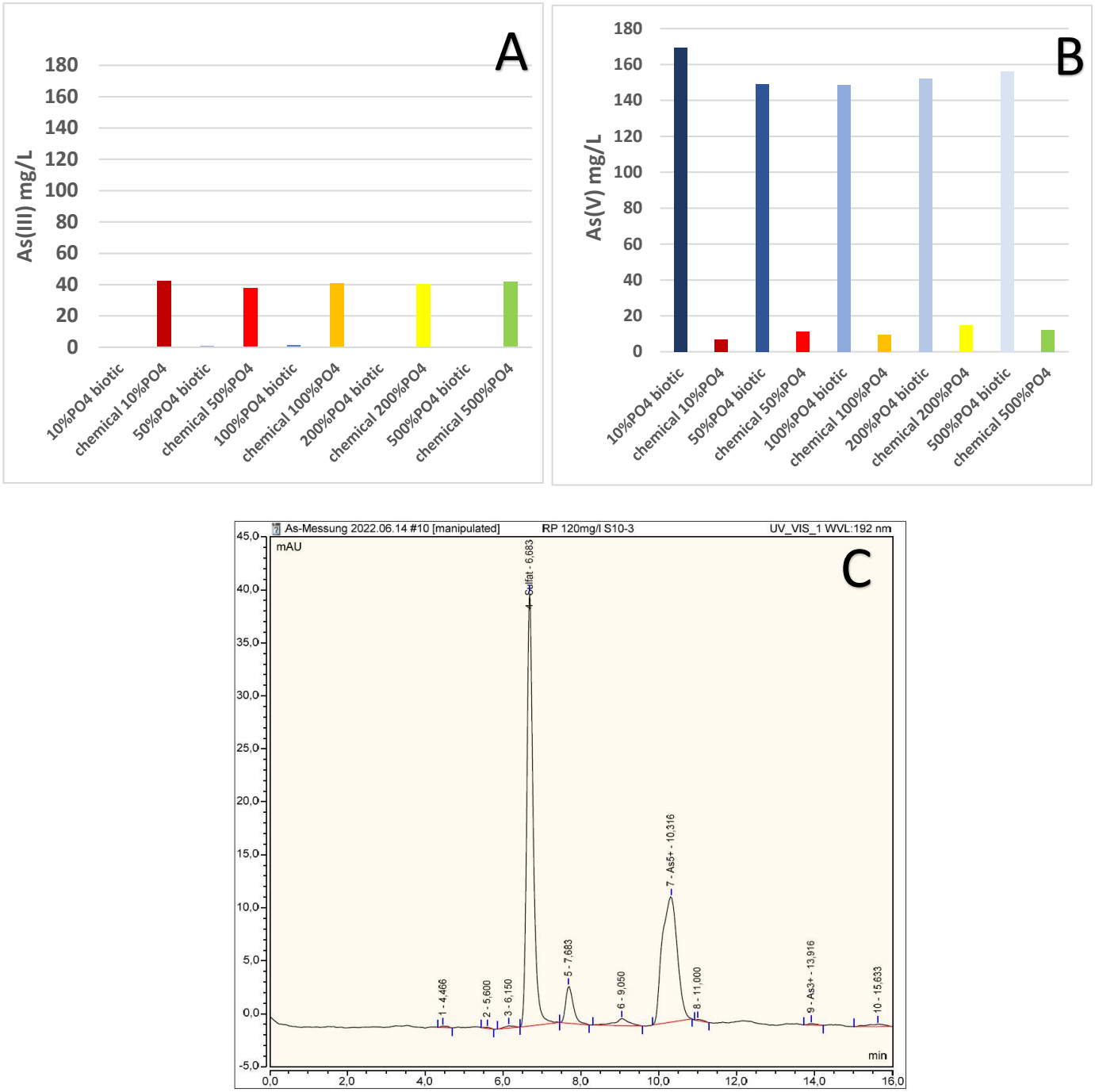
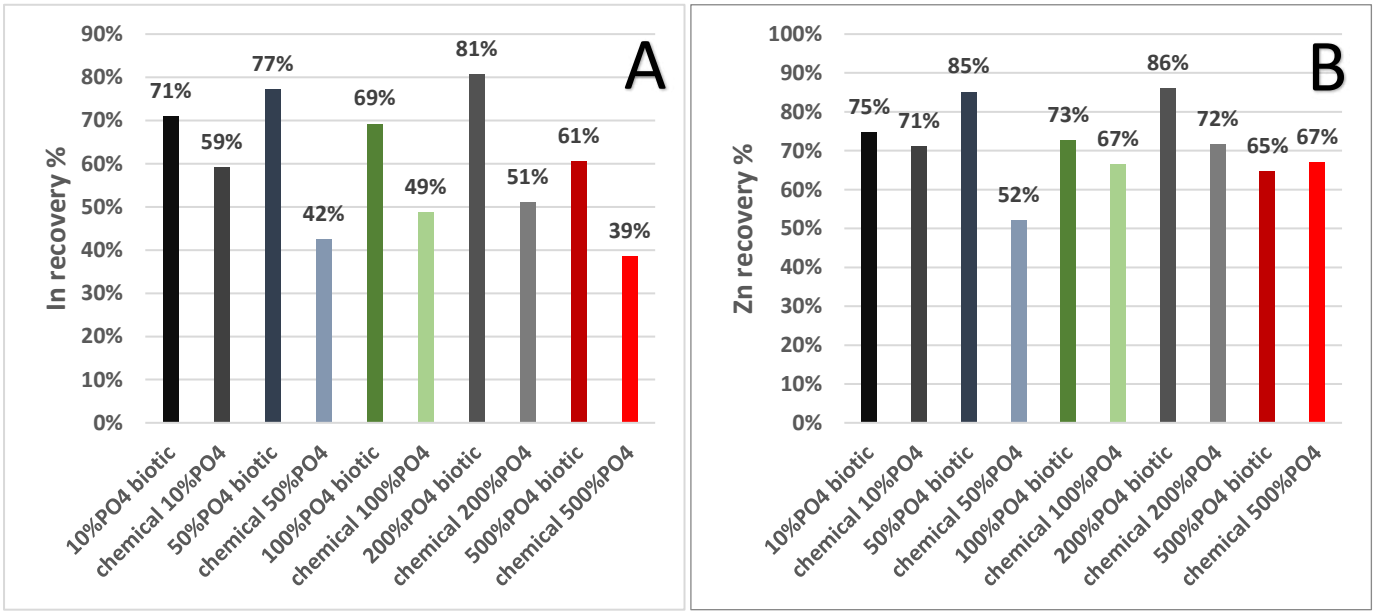
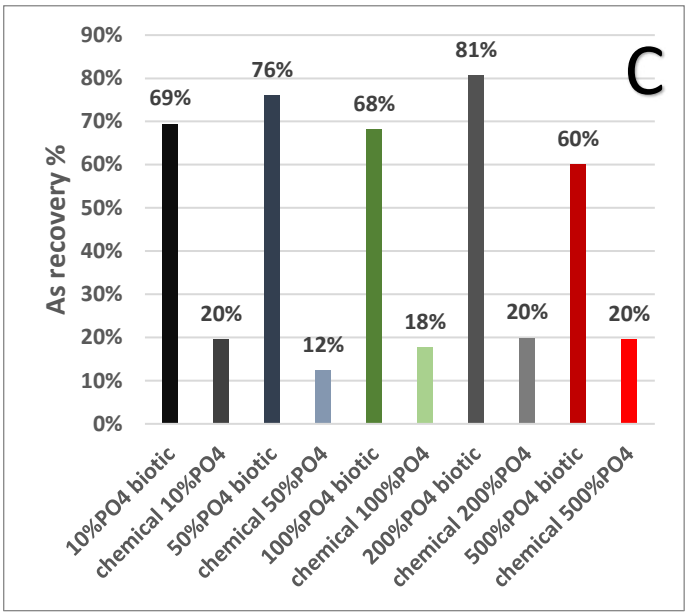


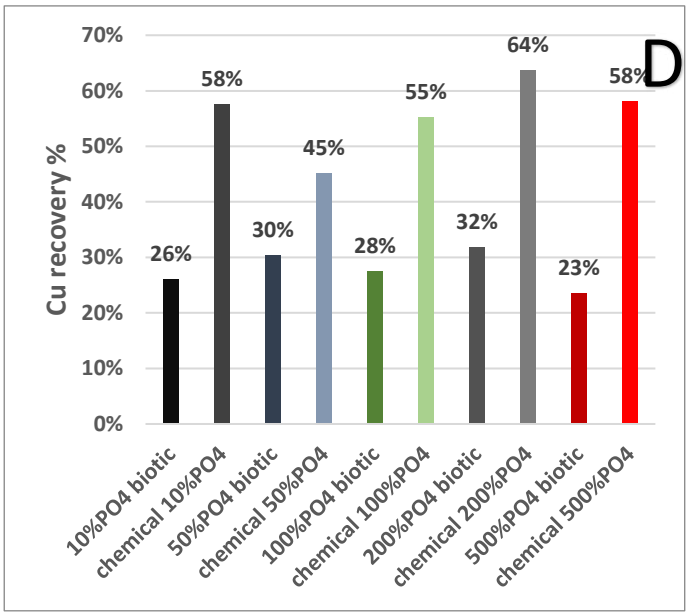
Figure 10:IC measurement for identifying the species of As in the PLS of the bioleaching experiment using different concentration of phosphate (10%,50%,100%,200% and 500%).[A] shows the concentration of As(III) while [B] shows concentration of As(V) [C] Peak integration report for experiment biotic 10%PO4 showing the interference in the result of As(V) concentration. The measurement was made for samples taken on last day of the experiment

The recovery of both In (81%) and Zn(86%) is highest for the biotic 200%PO4.(Fig 10A&B).The biotic experiments have higher recovery of In(60-81%) as compared to abiotic (39-59%) which is also the case for Zn as well. Since the bacteria oxidises the Fe(II) to Fe (III) which is the main oxidant for dissolution of the metal sulphides, thereby promoting the dissolution of In and Zn in the PLS ((Deveci et al. 2004). (Fig 12 A&B) shows that almost all the Fe (II) has been

oxidised in the biotic experiments. The pH (Fig 8 A&B) at day 21 reaches nearly 2.5 for the abiotic while the biotic have low pH 1.8. The redox (Fig 8 C&D) is < 380mV in the chemical controls while the biotic have high redox >500mV. Therefore, the biotic have favourable condition for higher solubility and oxidation which results in higher recovery of In and Zn. It had been found that the pH plays a significant role in the bacterial activity of moderate thermophilic bacteria and for precipitation of ferric iron mainly as K-jarosite (Deveci et al. 2004). The highest yield of In and Zn by 200%PO4 biotic could be due to the higher bacterial growth (Fig 7) compared to other PO4 concentrations. Unexpectedly, 500%PO4 biotic has the lowest yield of In and Zn, which could be explained by the formation of scorodite (which is absent for 200%PO4) and jarosite with relatively higher (vol%) (Table 2). The recovery of Cu (Fig 10D) is very low for the biotic experiments mainly due to the high redox. Since the Cu is present in the form of chalcopyrite, the formation and accumulation of scorodite and jarosite could form a passivation layer on the chalcopyrite which hampers the leaching of the Cu. (Deng et al. 2020b).Formation of sulphur and basic iron sulphate could also be the reason for the incomplete dissolution of Cu from chalcopyrite. (Keeling et al. 2005).







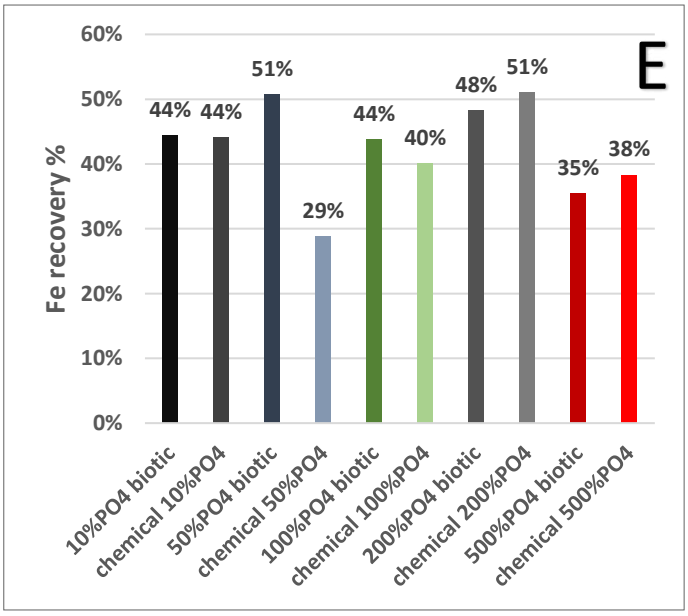


Figure 11: Recovery % of [A] In, [B] Zn, [C] As , [D] Cu,[E] Fe in the PLS after 21 days of bioleaching experiment of 1% solid load of Pöhla concentrate using Sulfobacillus thermosulfidooxidans where inoculation is done in MAC medium containing different phosphate concentration 10%,50%,100%,200% and 500% (corresponding to the normal concentration of phosphate in MAC medium). Addition of 50mM Fe (II) and 0.02% yeast extract before inoculation.

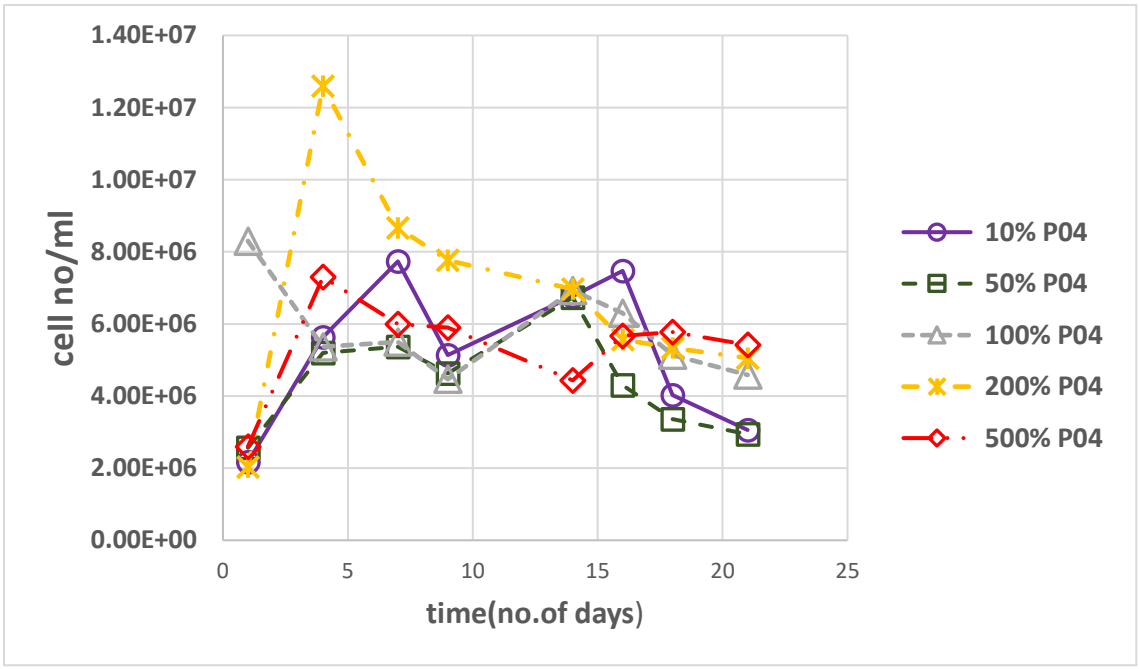
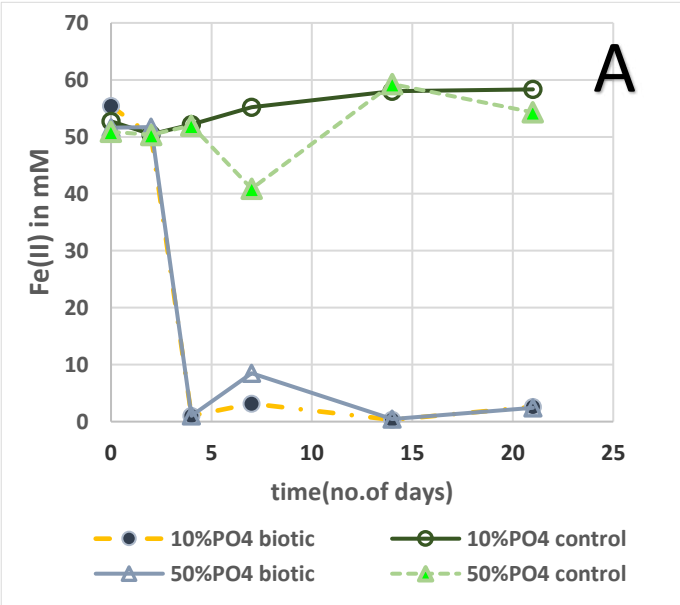
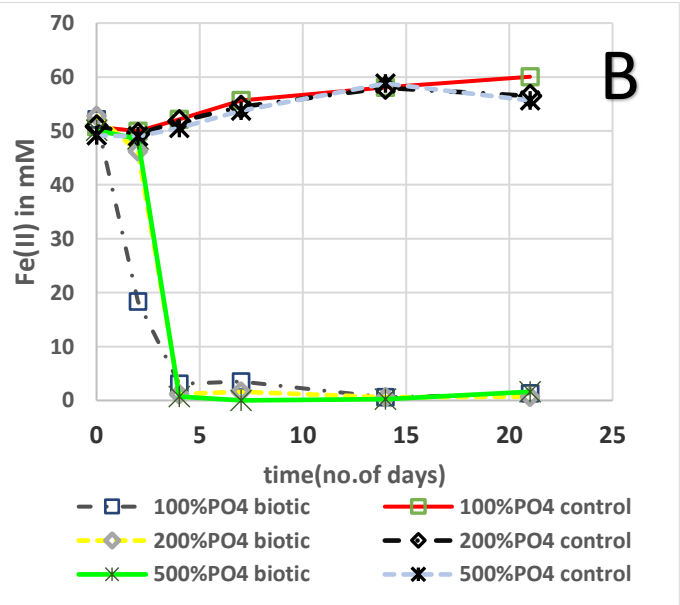


Figure 12: Cell counting for bioleaching experiment of Pöhla concentrate(1%SL) using Sulfobacillus thermosulfidooxidans inoculated in MAC medium containg different concentration of PO4 10%,50%,100%,200%&500% (corresponding to the normal PO4 concentration in MAC m





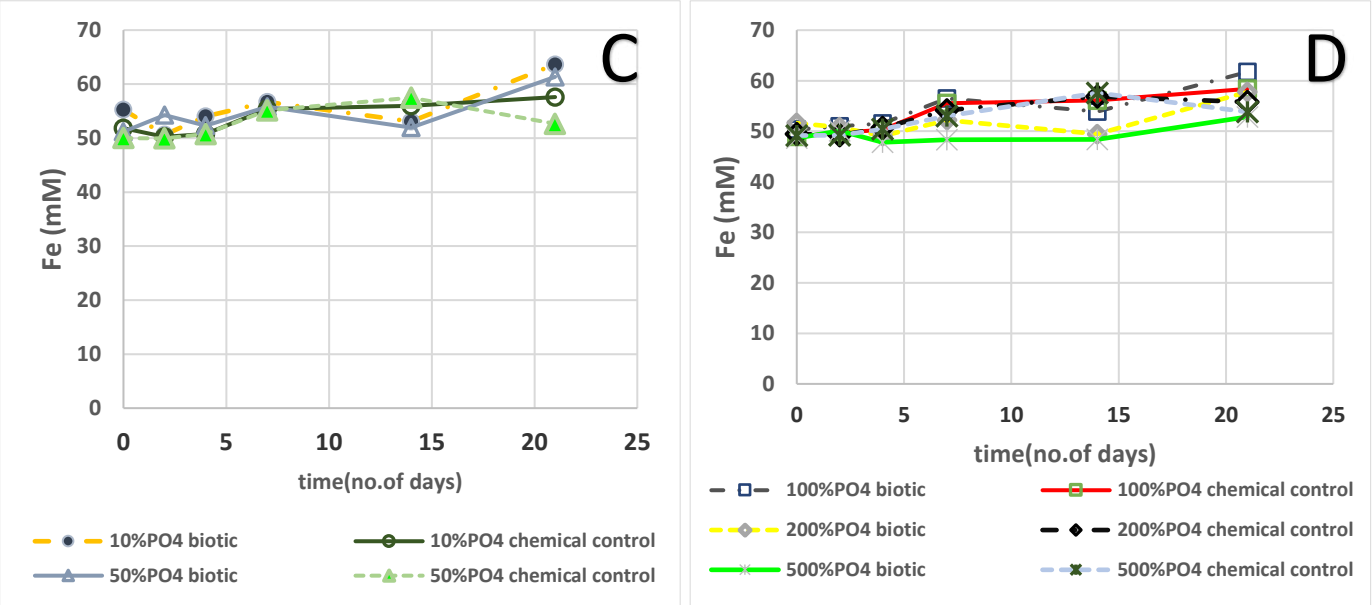
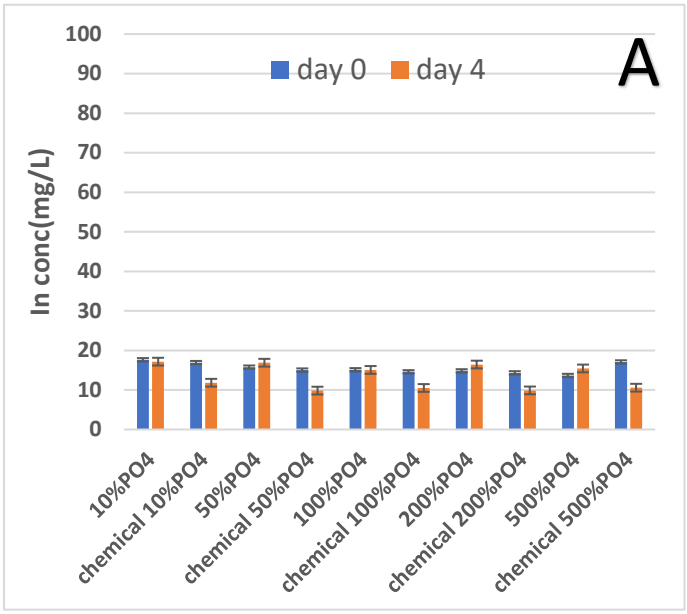
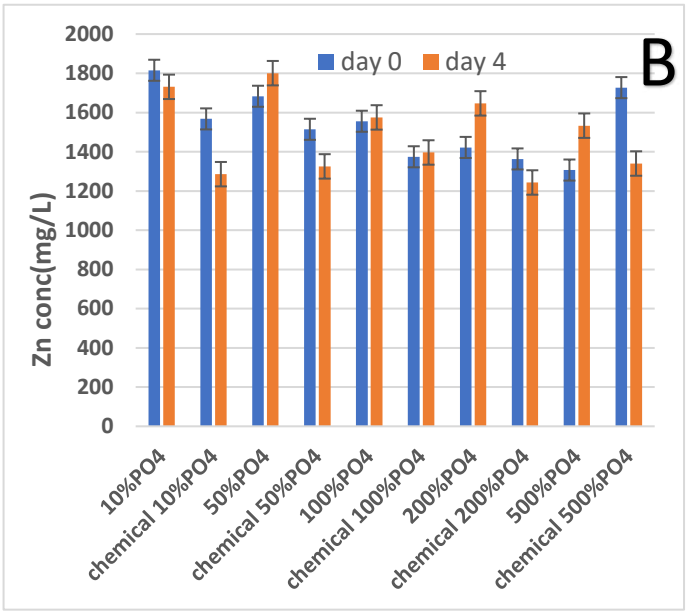


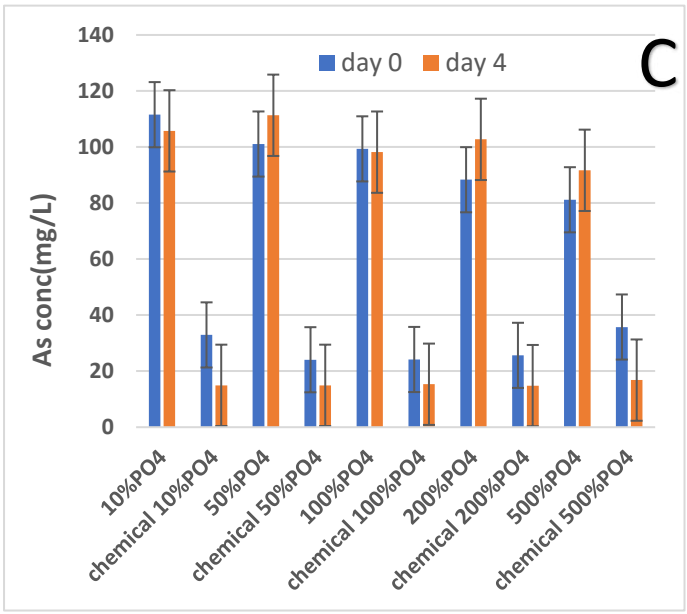
Figure 13: Fe(II)[A&B] and total Fe [C&D] concentration measured by ferrozine method for bioleaching experiment of Pöhla concentrate(1%SL) using Sulfobacillus thermosulfidooxidans inoculated in MAC medium containing different concentration of PO4 [A]10% & 50% and [B]100%,200% for 21 days.

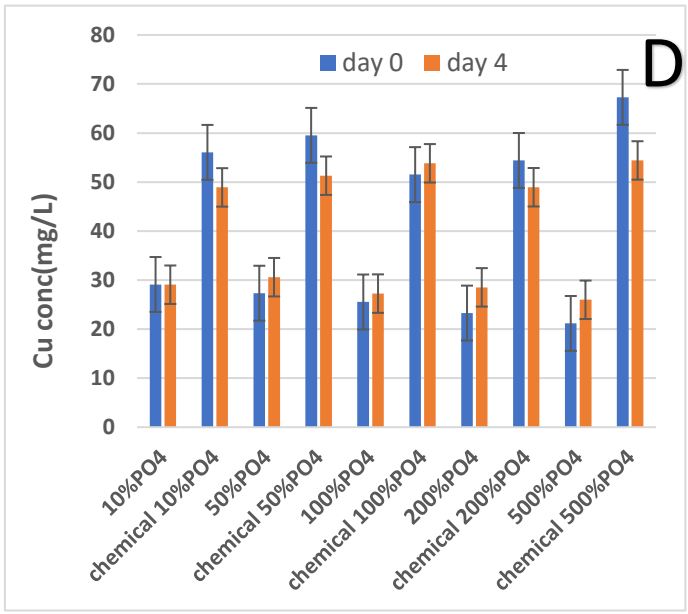
Table 2 Result of XRD analysis for solid residue collected after 25 days of the bioleaching experiment of the Pöhla concentrate (1%) using Sulfobacillus thermosulfidooxidans inoculated in MAC medium containing different phosphate concentration. For this analysis, 500% biotic and 200%biotic PO4 are analyse as they have the highest and lowest In recovery respectively among the biotic experiments.

	quartz SiO_2	**chalcopyrite** $CuFeS_2$	**sulphur** S	**spinel (magnetite)** AB_2O_4	**hematite** Fe_2O_3	**scorodite** $FeAsO_4$	**jarosite** $KFe_3OH_6(SO_4)_2$	**hornblende** $(Ca,Na)_{2-3}(Mg,Fe,Al)_5(Al,Si)_8O_{22}(OH,F)_2$
500%PO4 biotic (Vol %)	20 ± 2	~ 1	21 ± 2	~ 1	5 ± 1	2 ± 1	34 ± 3	16 ± 2
200%PO4 biotic (Vol %)	21 ± 2	~ 1	20 ± 2		4 ± 1		31 ± 3	23 ± 2


A
day 0
day 4
In conc(mg/L)
100
90
80
70
60
50
40
30
20
10
0
10%PO4
chemical 10%PO4
50%PO4
chemical 50%PO4
100%PO4
chemical 100%PO4
200%PO4
chemical 200%PO4
500%PO4
chemical 500%PO4


B
day 0
day 4
Zn conc(mg/L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10%PO4
chemical 10%PO4
50%PO4
chemical 50%PO4
100%PO4
chemical 100%PO4
200%PO4
chemical 200%PO4
500%PO4
chemical 500%PO4


C
day 0
day 4
As conc(mg/L)
140
120
100
80
60
40
20
0
10%PO4
chemical 10%PO4
50%PO4
chemical 50%PO4
100%PO4
chemical 100%PO4
200%PO4
chemical 200%PO4
500%PO4
chemical 500%PO4


D
day 0
day 4
Cu conc(mg/L)
80
70
60
50
40
30
20
10
0
10%PO4
chemical 10%PO4
50%PO4
chemical 50%PO4
100%PO4
chemical 100%PO4
200%PO4
chemical 200%PO4
500%PO4
chemical 500%PO4

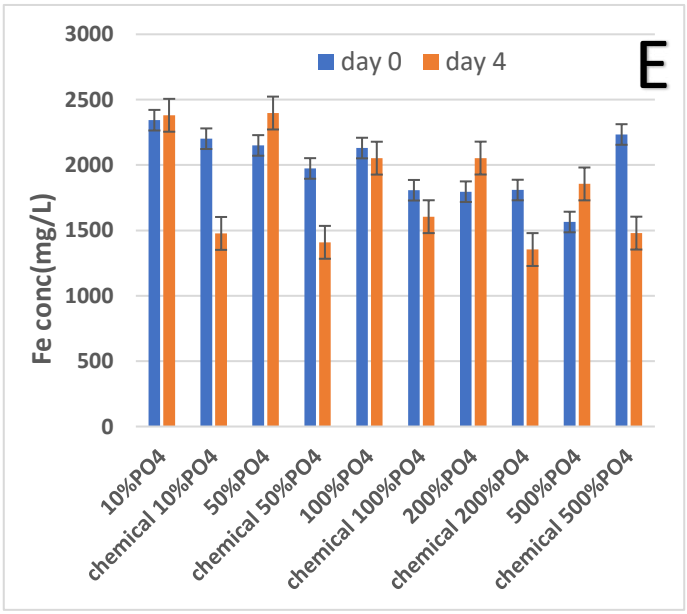

E
day 0
day 4
Fe conc(mg/L)
3000
2500
2000
1500
1000
500
0
10%PO4
chemical 10%PO4
50%PO4
chemical 50%PO4
100%PO4
chemical 100%PO4
200%PO4
chemical 200%PO4
500%PO4
chemical 500%PO4

Figure 14 : After day 21 of the bioleaching experiment using different concentration of phosphate,0.00185 mg/L of In (in the form of $InCl_3$) is added to all the flasks and incubated again for 4 days. [A] In,[B] Zn, [C] As, [D] Cu [E] Fe shows the change in concentration (ICPMS measurement) from day 0 compared to day 4.

To find out the possible interaction between In and PO_4^{3-} in the bioleaching medium, 0.00185 mg/L of In were added to the experiments on day 21 and continue for 4 days. We can see that, after day 4, the In concentration does not change much in the biotic experiments but for the chemical controls, the In concentration decreases suggesting precipitation of In. With this being said, there are no significant differences among different PO_4^{3-} concentration indicating that PO_4^{3-} is not the precipitating agent.

Overall, we can conclude that the PO_4^{3-} have not much influence on the recovery of the metals In and Zn. The difference in growth rate of the bacteria in the different PO_4^{3-} concentrations (Fig 11) which could be a factor for the difference in the yield among the different PO_4^{3-} concentration. The pH, redox and Fe^{2+}/Fe follow almost identical changes for all the PO_4^{3-} concentrations. The inhibition of recovery of the metals could be the result of formation of secondary minerals like elemental S and jarosite whose formation in substantial amount is confirmed by the XRD analysis result (Table 2).

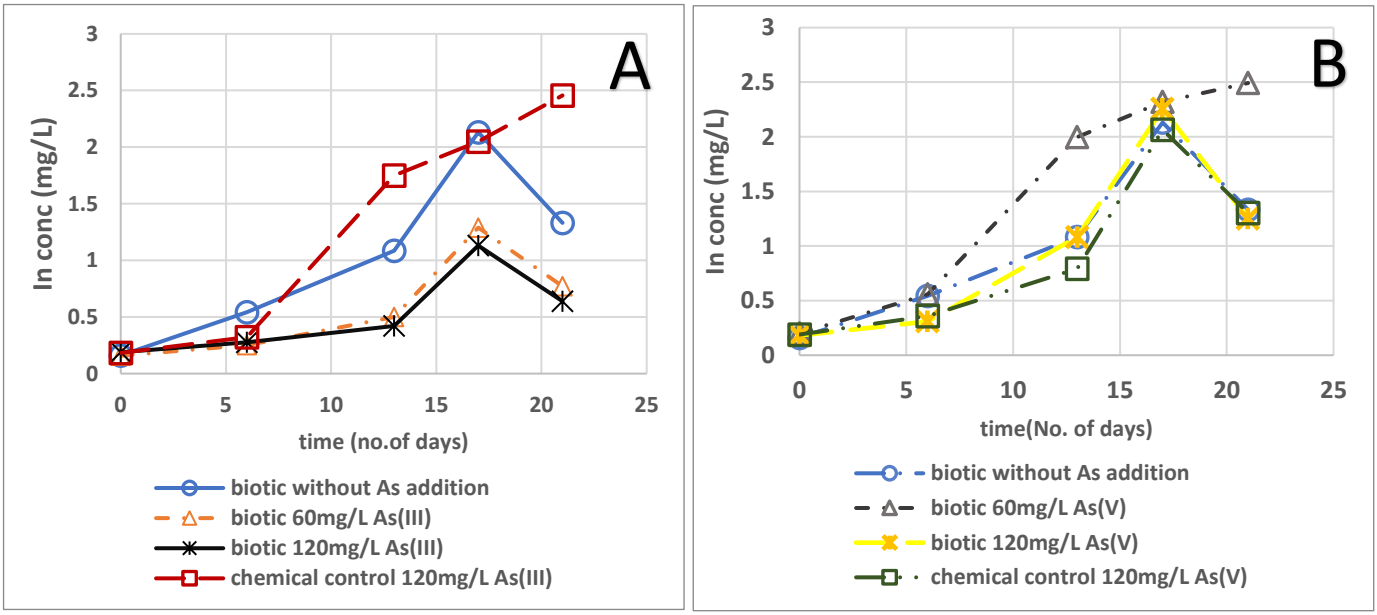
3.2 Bioleaching using different arsenite and arsenate concentration

To have an insight on the effect of As on bioleaching recovery of In, the Pöhla concentrate was leached using *Sulfobacillus thermosulfidooxidans* with different concentrations (60 mg/L & 120 mg/L) of As (III) and As (V) for 21 days. (Fig 15 A,B,C&D) shows that the concentration in PLS of In and Zn escalated after day 6 for all the experiment and reaches their respective peak concentrations on day 17 except C(III)120 & B(V)60 which increases further till day 21 while the rest of the experiments decreases after day 17 (Fig 15 A,B,C&D) The pH for the biotic experiments where As(III) and As(V) follows the same trend and was < pH2 (Fig 17 A&B) during the experiment while the chemical controls had a slightly higher pH and reaches nearly pH 2.5 on the 14th day. It should be noted that between day 10 and day 15, pH control was not added which is why the pH was high in the chemical controls. The experiment where As (III) was added, the redox (Fig 17 C&D) for the biotic and chemical control were similar <380mV and does not increase over time. Whereas in the experiment where As (V) was added, the biotic ones had a higher redox which increase from day 7 upto 570 mV and remain stable until the last day. The chemical control C(V)120 forms a straight line and <390 mV throughout the experiment. From (Fig 18), we can see that the As [both As (III) and As(V)] causes inhibition to the cell growth when compared with that of the biotic control. Although As(V) added

experiment has comparatively higher growth rate than the As (III) added ones which could be explained by the Fe (II) concentration (Fig 19 A&B) where bacteria with As (III) added was not able to oxidised the Fe (II).

The As dissolution (Fig 15 E&F) for biotic and abiotic of the As (III) added experiments show similar curve which decline at the beginning and then accelerate until day 17 and then decreases again from then. Whereas in experiment with added As (V) the chemical control has the lowest concentration of As. The biotic B(V)60 and B(V)120 both increases with time and reaches their respective highest on day 21(252.2 mg/L) and day 17 (268.2 mg/L). (Fig 14 E&F) As expected the concentration of Cu in the PLS (Fig 16 A&B)) is higher in the chemical control than the biotic ones for both the experiment with added As (III) and As (V) with the exception of B(V)60. The Fe concentration (Fig 16 C&D) remains constant till day 13 and then rises to > 2500 mg/L on day 17 and then decreases again on day 21. This trend is followed with the exception of chemical control 120 mg/L As (III) and biotic 60 mg/L As(V) where the Fe concentration increases from day 6 and reaches on day 13 (4180 mg/L) and on day 21 (2854.3 mg/L).

The concentration of In, Zn, As, Cu and Fe in the PLS all decreases from day 17 to day 21 in all the experiment except C(III)120 and B(V)60 which is quite abnormal. It is hard to find any connections with other results which may result in this decrease and thus remain inconclusive.



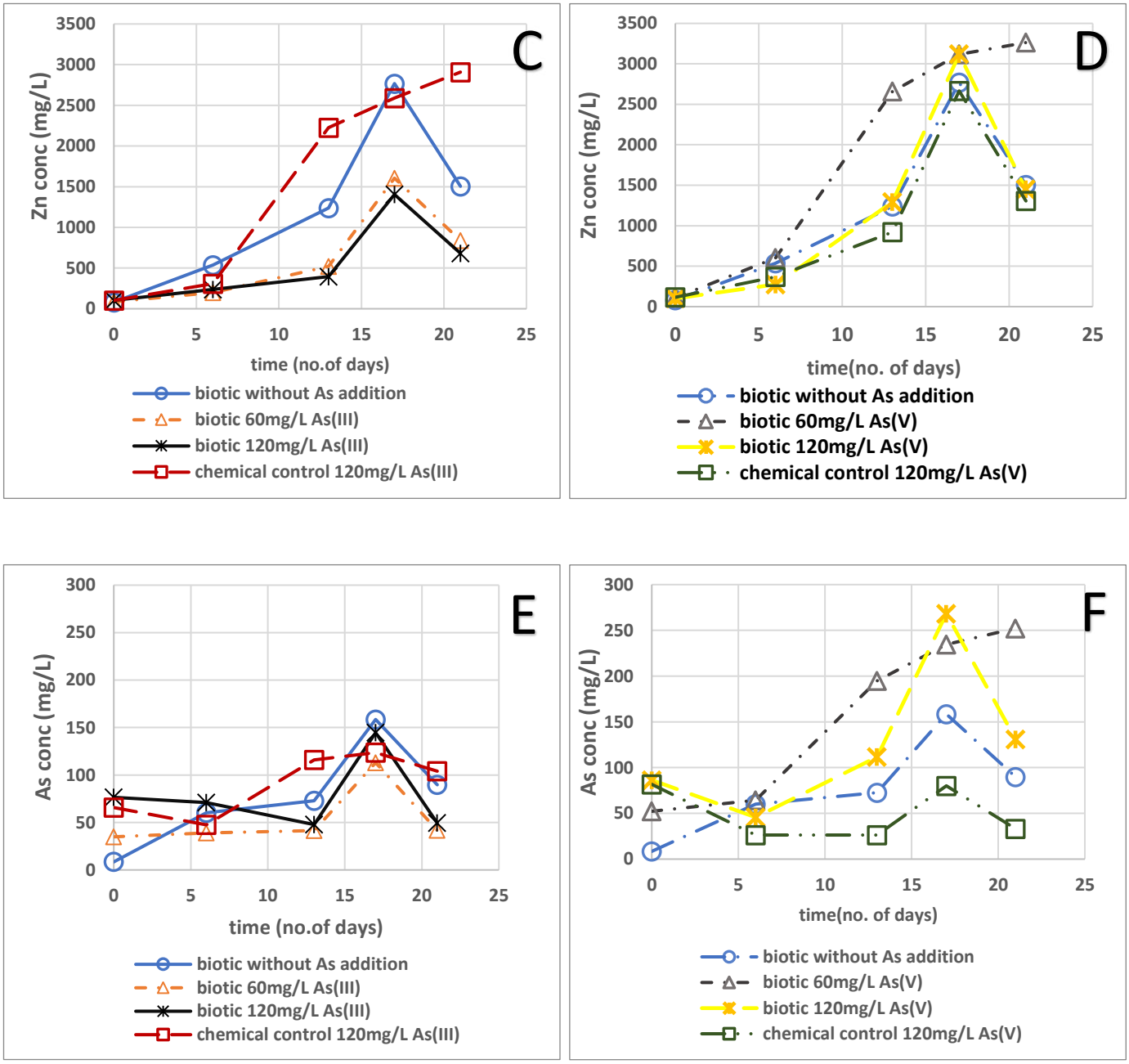


Figure 15: ICPMS measurement of concentrations (mg/L) of In[A&B], Zn[C&D], As[E&F] in PLS collected from bioleaching experiments of the Pöhla concentrate 1% solid load for 21 days using Sulfobacillus thermosulfidooxidans with different concentrations (60 mg/L &120 mg/L) of As(III)] and As(V).

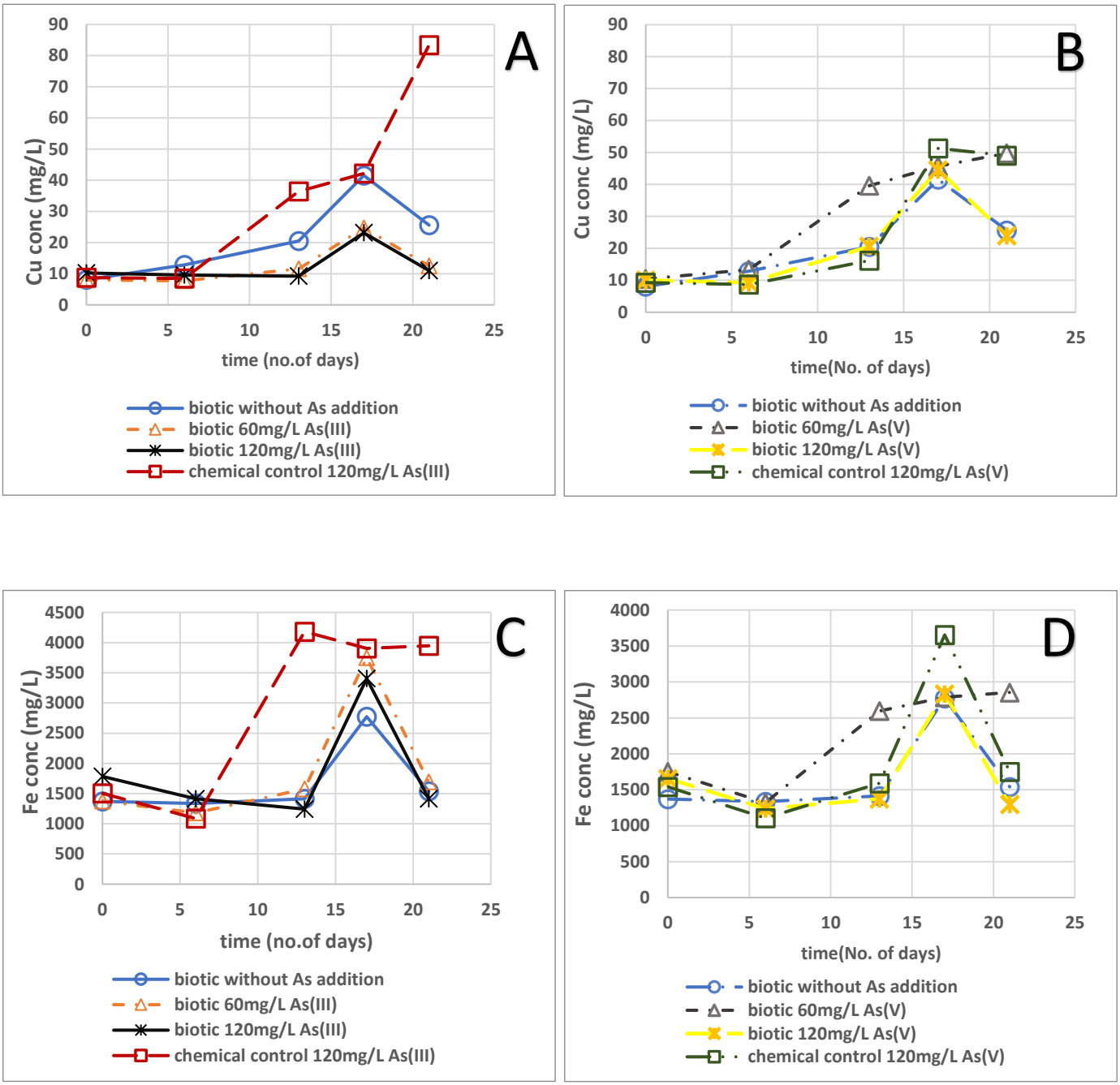


Figure 16: ICPMS measurement of concentrations (mg/L)] of Cu[A&B] and Fe[C&D] in PLS collected from bioleaching experiments of the Pöhla concentrate 1% solid load for 21 days using Sulfobacillus thermosulfidooxidans with different concentrations (60 mg/L &120 mg/L) of As(III)] and As(V).

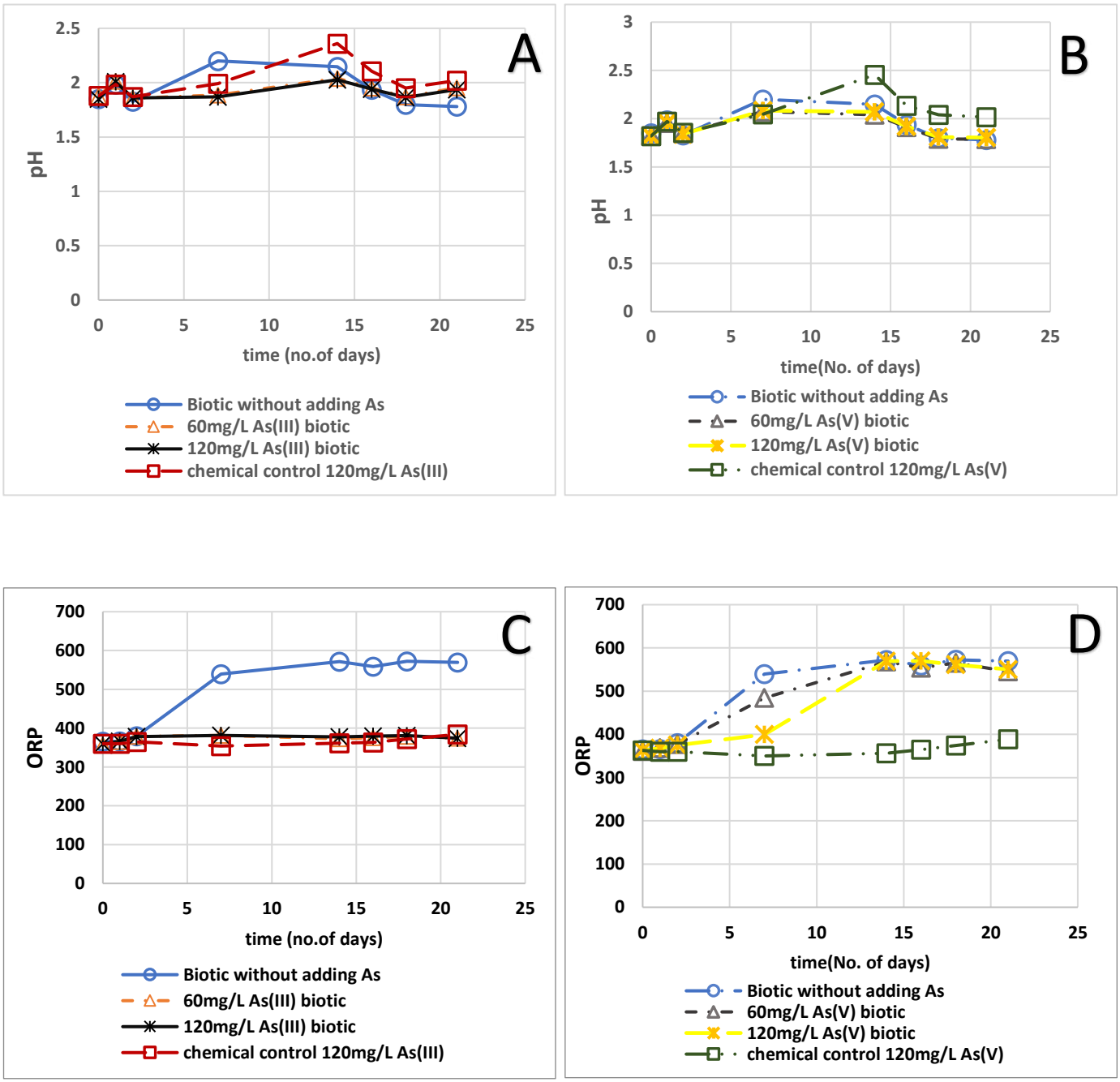


Figure 17: pH [A&B] and ORP [C&D] measurements during bioleaching experiment of Pöhla concentrate (1%SL) using Sulfobacillus thermosulfidooxidans for 21 days with different concentrations (60mg/L & 120mg/L) of As (III) and As(V) being added. The pH 1.8 was regulated using 95% H_2SO_4 (except day 10 to 15).

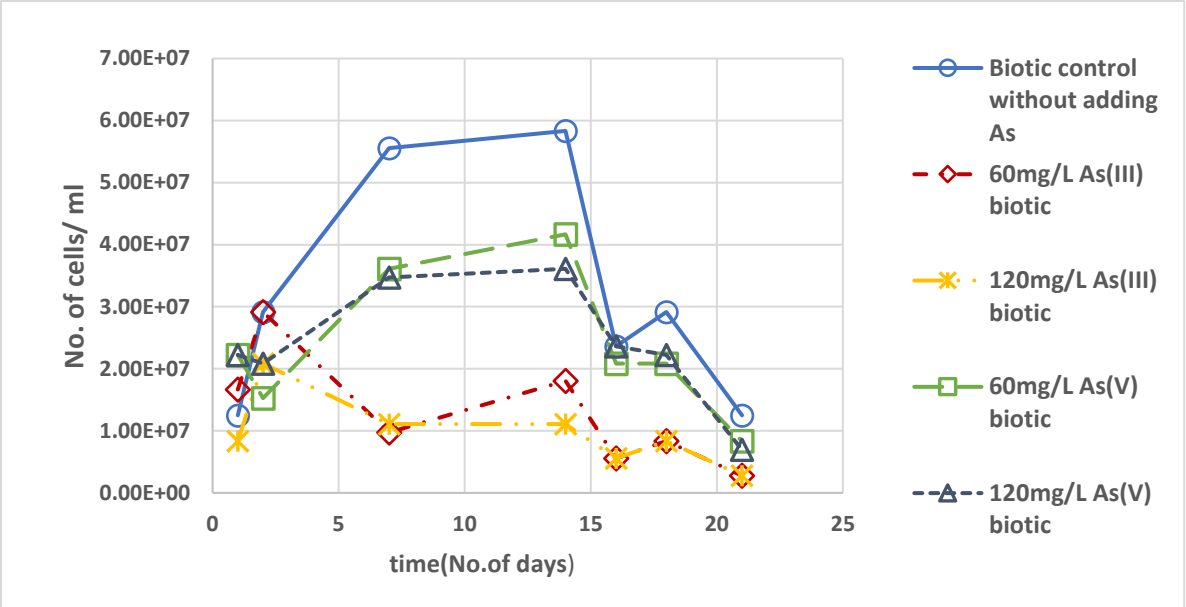
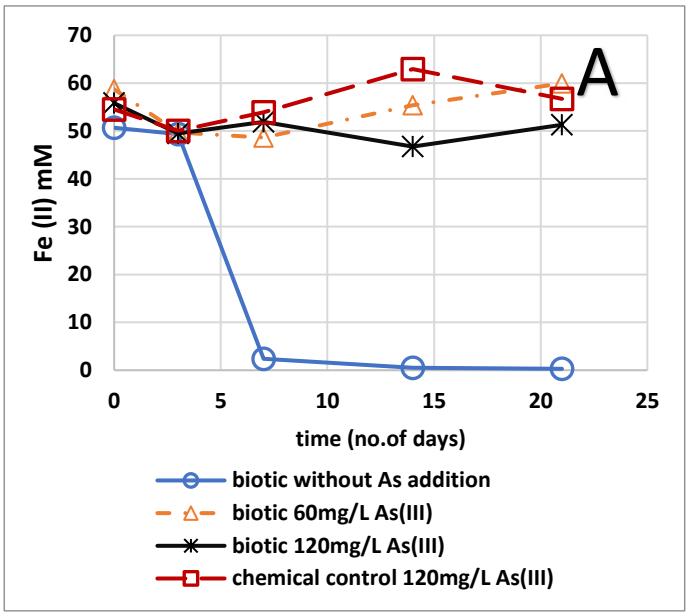
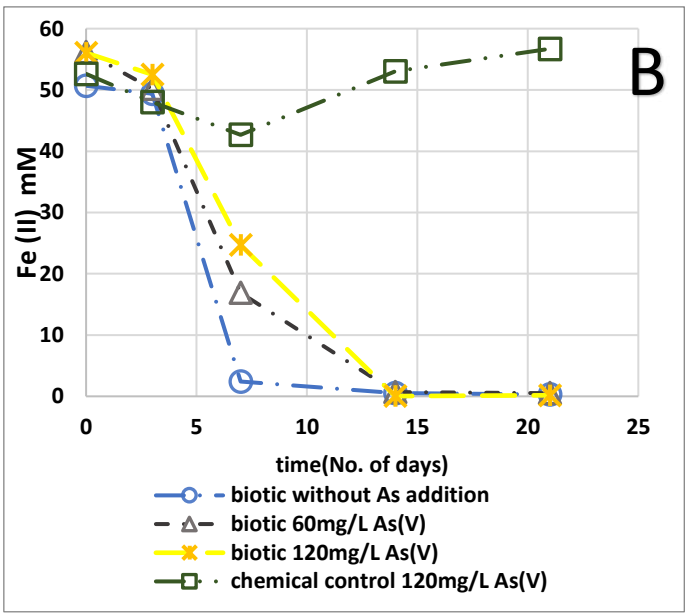


Figure 18: Cell counting of Sulfobacillus thermosulfidooxidans after 21 days bioleaching experiments of the Pöhla concentrate (1% SL) inoculated in MAC medium ,50mM Fe(II),0.02% yeast extract and with different concentrations (60mg/L & 120mg/L) of As(III) and As(V) being added





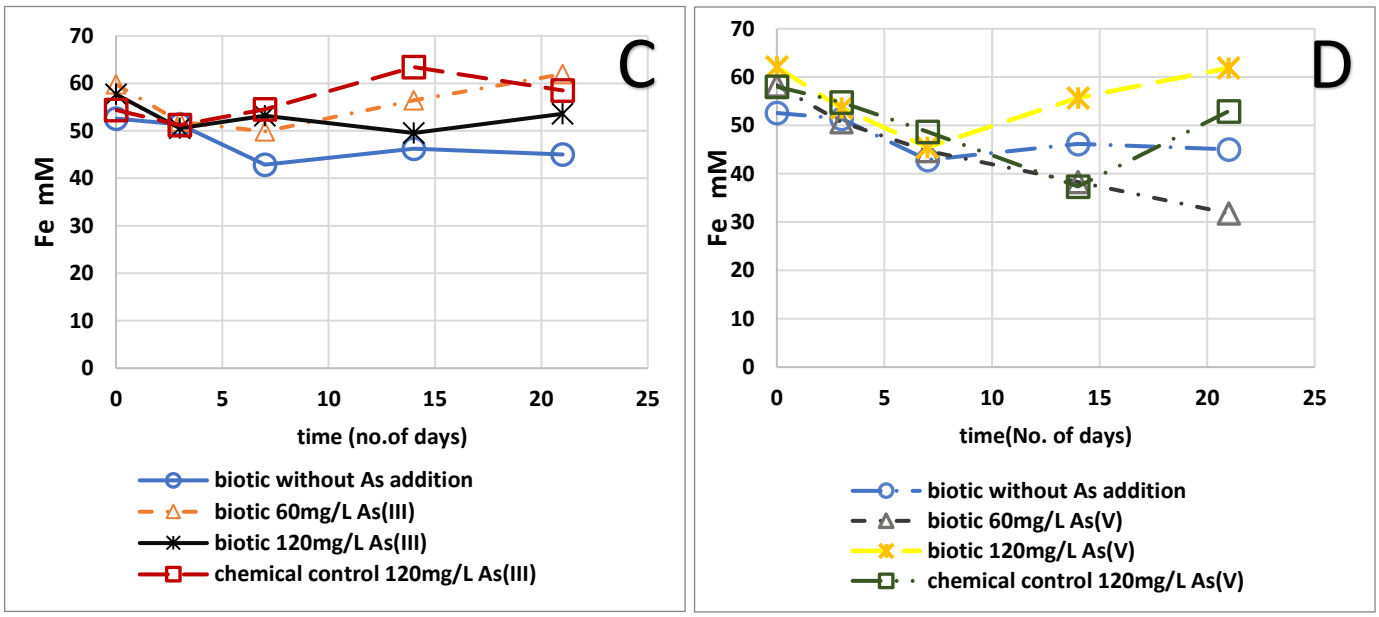
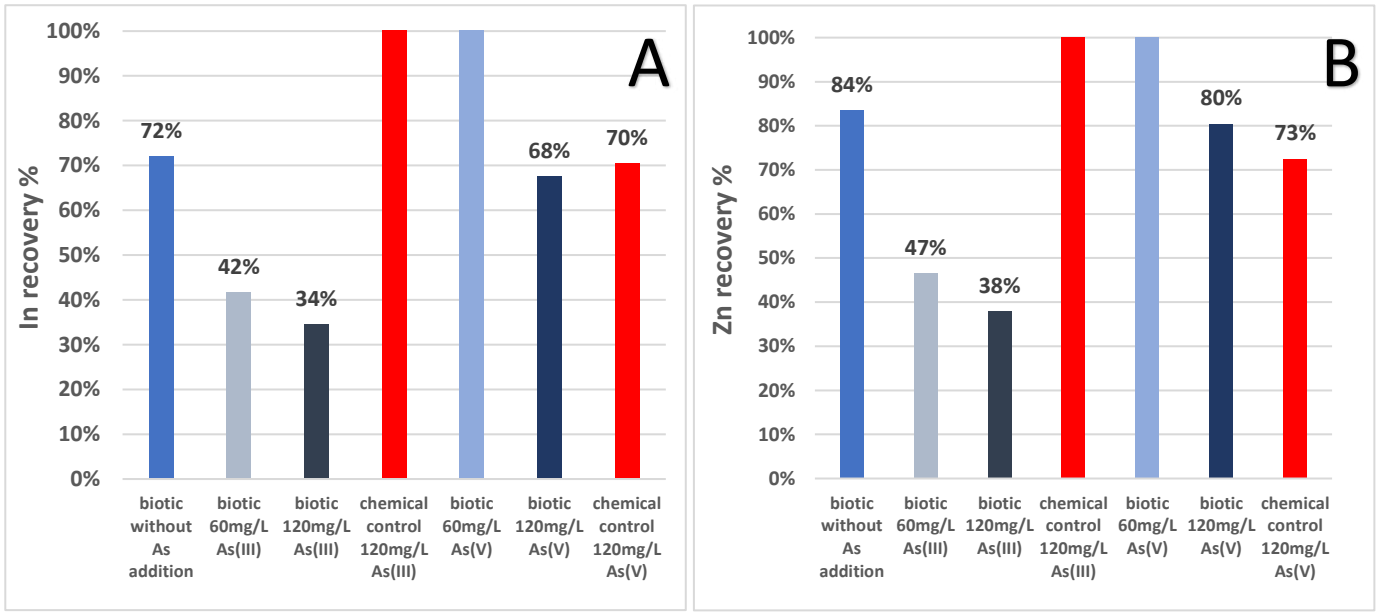


Figure 19: Measurement of Fe(II)[A&B] and total Fe [C&D] by ferrozine method for bioleaching experiment of (1%SL) Pöhla concentrate using with different concentrations (60mg/L & 120mg/L) of As(III) and As(V)



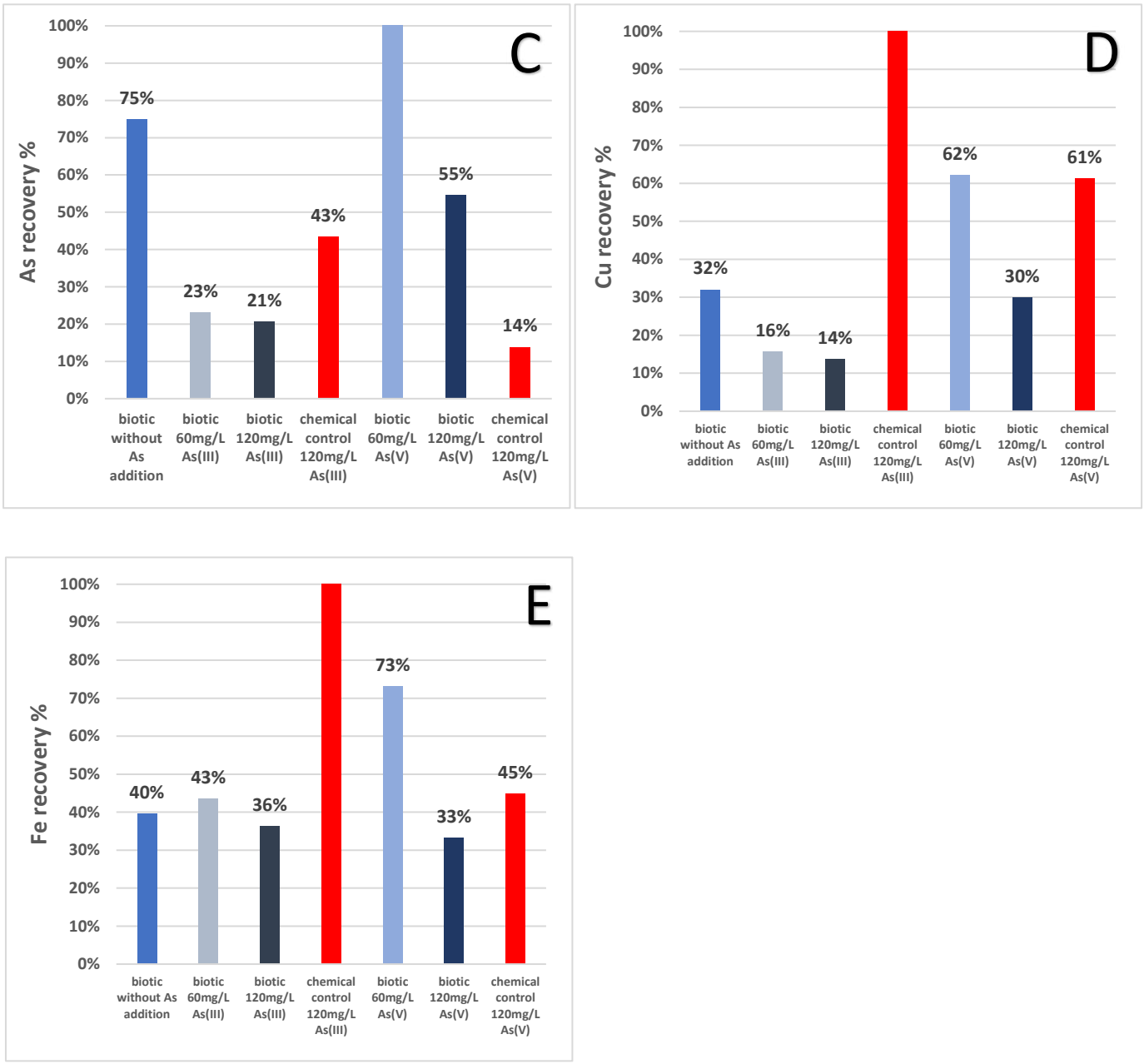
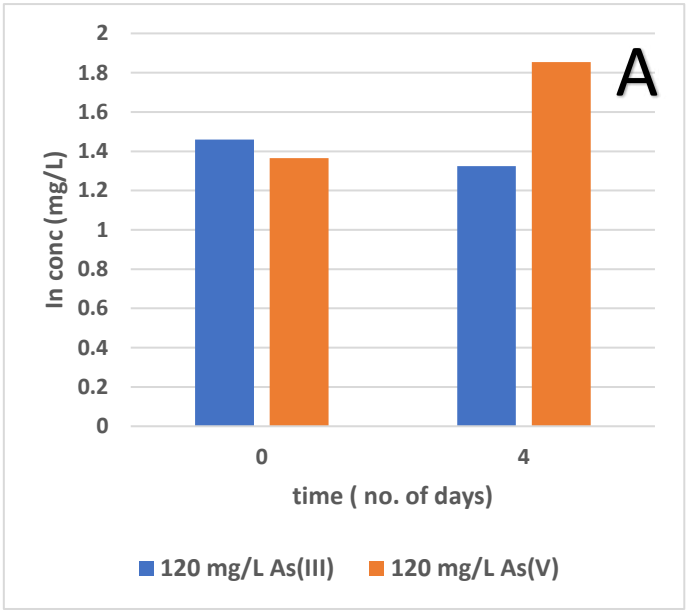


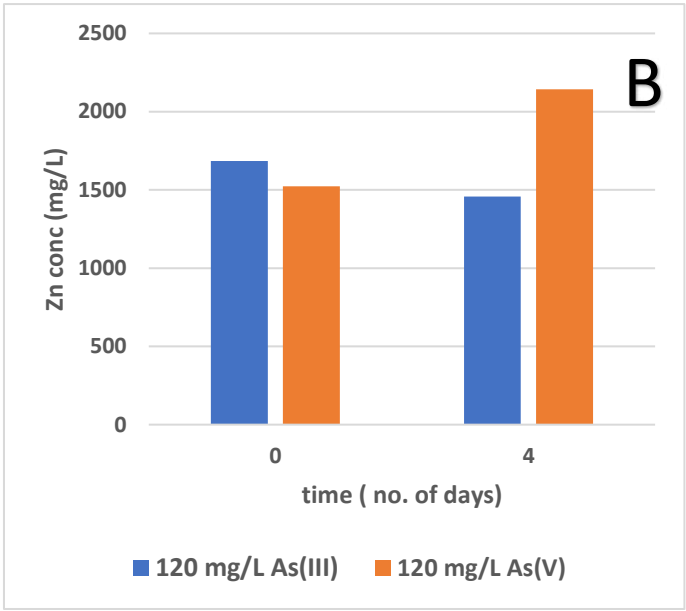
Figure 20: Recovery % of [A] In, [B] Zn, [C] As, [D] Cu and [E]Fe in the PLS after 21 days bioleaching experiments of the Pöhla concentrate 1% solid load using Sulfobacillus thermosulfidooxidans. The experiment is carried out for different concentrations 60mg/L& 120mg/L of As(III) and As(V) separately at 50°C with pH 1.8 maintained manually using 95% H_2SO_4

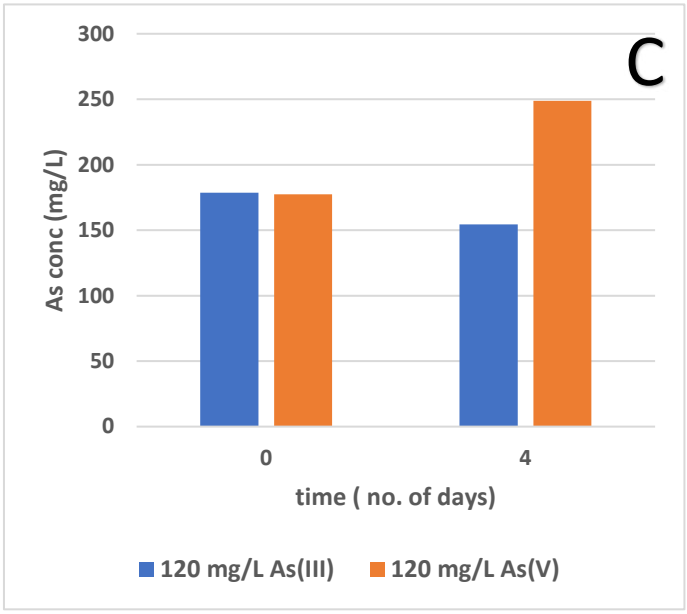
After 21 days of bioleaching experiment using different As (III) and As (V) concentration, 100% recovery of In (Fig 20A) and Zn (Fig 20B) from the concentrate could be achieved by C(III)120 and B(V)60 respectively. Comparing the recovery of In and Zn between the B(V)120 (68% In & 80% Zn) and the biotic control experiment without added As (72% In and 84% Zn), we could see that there is no significant difference. As mentioned before, (Fig 18) shows that the As causes inhibitory to the cell growth yet B(V)60 has 100% In and Zn yield. This had led to a question whether As (V) could promote the dissolution of In and Zn from the sulphides. On the other hand, the biotic experiment with added As (III)- B(III)60 & B(III)120 can acquire only 42% & 34% of In and 47%& 38% of Zn (Fig 18 A&B). The biotic B(V)120 have lower recovery of In (68%) and Zn (80%) compared to that of the biotic control and the B(V)60 mostly because of the formation of substantial amount (50% vol) of jarosite. (Table 3). Jarosite is undesirable as it depletes the Fe (III) which hinders the dissolution of the metal sulphides by Fe (III)'s oxidative attack. Along with this, In can precipitate with jarosite as well resulting in the low recovery of In in the PLS (Wanjiya et al. 2015; Gunn 2014; A.M. Alfantazi and R.R. Moskalyk 2003). When As(V)/ Fe(III) molar ratio increases, the formation of ferrihydrite increases and crystallization of hematite is favoured (Violante et al. 2007) which can be confirmed by the presence of hematite is present in the solid residue collected from the experiments(Table 3).

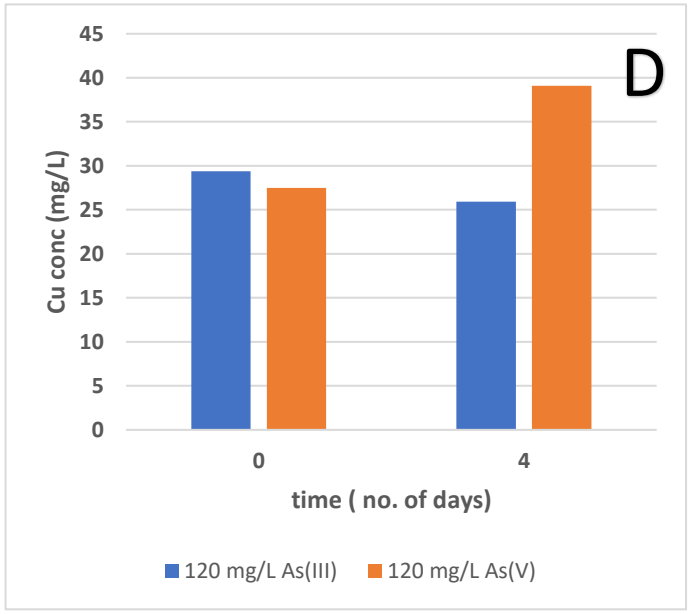
100% of As (Fig 18C) in the medium was recovered in experiment B(V)60 while only 14 % from experiment C(V)120. The recovery of As is very low for experiment with added As(III) all < 40%.100% of Fe (Fig 18E) and Cu (Fig 18D) were recovered in the experiment C(III)120.Only 14% of Cu is recovered B(III)120 experiment which can be explained by the presence of ZnS in the solid residue shown by the XRD result(Table 3).This particular experiment is interesting because there were no scorodite or jarosite formation but have 29% vol of sulphur shown by the XRD result. This signify that the low recovery of Cu (14%) could be the effect of formation of sulphur along with the inability of the bacteria to oxidise Fe(II) to Fe (III) (Fig 17).It has been reported that the passivation during bioleaching of Cu from chalcopyrite is mainly caused by elemental sulphur. (Fu et al. 2012).

The higher In yield (100%) by the experiment B(V)60 and C(III)120 could be attributed to the high recovery of Fe 73% &100% respectively in the PLS. This could result in low formation of secondary iron precipitate mainly jarosite which as mentioned above could easily inculcate In with it.


A
In conc (mg/L)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
4
time (no. of days)
120 mg/L As(III)
120 mg/L As(V)


B
Zn conc (mg/L)
2500
2000
1500
1000
500
0
0
4
time (no. of days)
120 mg/L As(III)
120 mg/L As(V)


C
As conc (mg/L)
300
250
200
150
100
50
0
0
4
time (no. of days)
120 mg/L As(III)
120 mg/L As(V)


D
Cu conc (mg/L)
45
40
35
30
25
20
15
10
5
0
0
4
time (no. of days)
120 mg/L As(III)
120 mg/L As(V)

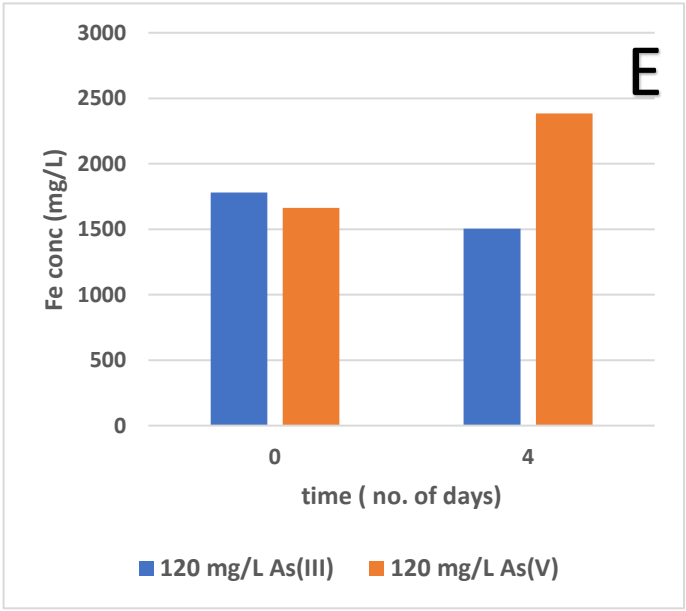

E
Fe conc (mg/L)
3000
2500
2000
1500
1000
500
0
0
4
time (no. of days)
120 mg/L As(III)
120 mg/L As(V)

Figure 21:Concentration of [A] In, [B] Zn, [C] As, [D] Cu and [E]Fe in the PLS after adding 120 mg/L of As(III) in one set of biotic control and 120 mg/L of As(V) to another set of biotic control at the end of the previous experiment where 1% Pöhla concentrate was bioleached using different concentrations of As(III) and As(V) fro 21 days.

After the 21st day of the experiment,120 mg/L of As(III) were added to the biotic control B1 and 120 mg/L of As (V). to another biotic control B2 (both triplicates) and was run for 4 more days. This was done in case the bacteria were not able to leached significant amount of the In from the concentrate when As was added to the experiment. Adding the As (III) or As(V) to the biotic control would enable us to know the interaction of As(III) or As(V) with the leached In in the bioleaching medium. After 4 days, we can see that the concentrations of In along with Zn, As, Cu and Fe all increases for B2 while that of the experiment with B1 decreases (Fig 19). The increase in In and. Zn concentration could be the result of the influence of the bacteria in further leaching the concentrate. The XRD analysis of the biotic control B1 and B2 shows no significant differences in the secondary precipitates formed. (Table 3).

Table 3: Result of XRD analysis of the solid residue collected at the end of bioleaching experiment from biotic 120 mg/L As (III) & biotic 120 mg/L As(V) biotic experiments and biotic control 1 and 2 (after adding As III and As V and run for 4 days).

	quartz SiO_2	**chalcopyrite** $CuFeS_2$	**sulphur** S	**spinel (magnetite)** AB_2O_4	**hematite** Fe_2O_3	**scorodite** $FeAsO_4$	**jarosite** $KFe_3OH_6(SO_4)_2$	**hornblende** $(Ca,Na)_{2-3}(Mg,Fe,Al)_5(Al,Si)_8O_{22}(OH,F)_2$	**diopside** $CaMgSi2O6$	**zinc blende** ZnS	**vermiculite** $Mg0,7(Mg,Fe,Al)6(Si,Al)8O20(OH)4\cdot 8H_2O$
120mg/L As(III) biotic [Vol%]	22 ± 3	2 ± 1	29 ± 3		5 ± 1			18 ± 2	6 ± 1	8 ± 1	10 ± 2
120mg/L As(V) biotic [Vol%]	13 ± 2	~ 1	17 ± 2		4 ± 1		50 ± 4	12 ± 2			3 ± 1
Biotic control 1 after adding 120 mg/L As(III) on day 21	14 ± 2	~ 1	23 ± 2		3 ± 1		45 ± 4	11 ± 2			4 ± 1

Biotic control 2 after adding 120 mg/L As (V) on day 21	16 ± 2	~ 1	17 ± 2		4 ± 1		46 ± 4	13 ± 2			4 ± 1

4. CONCLUSIONS

I. 1% is the optimum SL having the highest yield of In (68%) (Fig 1B) and Zn(80%) from the Pöhla concentrate while the other SLs>1% have significantly low recovery. The dissolution of silicate from the concentrate could be the main reason for the low recovery of In for SL>1%.

II. The formation of scorodite and jarosite could be main passivation in the bioleaching recovery of In from the Pöhla concentrate. This was seen in the experiment 200% and 500% PO4 where the In yield is higher in 200%PO4 (81%) than in 500%PO4 (61%). The XRD analysis result shows the formation of scorodite in 500%PO4 but not in 200%PO4 and jarosite is formed in both the experiment. The higher growth rate of *Sulfobacillus thermosulfidooxidans* in 200%PO4 could also be a factor for its higher In yield There was no concrete evidence for the direct influence of PO_4^{3-} on the recovery of In.

III. The bioleaching recovery of In by *Sulfobacillus thermosulfidooxidans* was not inhibited by the addition of As (V) (60 mg/L or 120 mg/L) where 100% & 68% of the In from the Pöhla concentrate could be recovered respectively. Whereas As(III) was toxic for the bacteria and substantial amount of In (<50%) was not able to be recovered in the biotic experiment with added As(III).The higher yield of In in experiment B(V)60 could be attributed to high recovery of Fe in the PLS and thereby low formation of jarosite.

5. REFERENCES

A.M. Alfantazi; R.R. Moskalyk (2003): Processing of indium: a review. In *Minerals Engineering* 16 (8), pp. 687–694. DOI: 10.1016/S0892-6875(03)00168-7.

Chung, Yong-Hwa; Lee, Chi-Woo (2012): Electrochemical behaviors of Indium. In *Journal of Electrochemical Science and Technology* 3 (1), pp. 1–13. DOI: 10.5229/JECST.2012.3.1.1.

Cook, Nigel J.; Ciobanu, Cristiana L.; Williams, Timothy (2011): The mineralogy and mineral chemistry of indium in sulphide deposits and implications for mineral processing. In *Hydrometallurgy* 108 (3-4), pp. 226–228. DOI: 10.1016/j.hydromet.2011.04.003.

Corkhill, C. L.; Vaughan, D. J. (2009): Arsenopyrite oxidation – A review. In *Applied Geochemistry* 24 (12), pp. 2342–2361. DOI: 10.1016/j.apgeochem.2009.09.008.

Deng, Sha; Gu, Guohua; Wu, Ziteng; Xu, Xiongyi (2017): Bioleaching of arsenopyrite by mixed cultures of iron-oxidizing and sulfur-oxidizing microorganisms. In *Chemosphere* 185, pp. 403–411. DOI: 10.1016/j.chemosphere.2017.07.037.

Deng, Sha; He, Guoshuai; Bichao, Wu; Guohua, Gu (2020a): Pyrite-promoted dissolution of arsenopyrite in the presence of Sulfobacillus thermosulfidooxidans. In *Journal of Materials Research and Technology* 9 (4), pp. 9362–9371. DOI: 10.1016/j.jmrt.2020.05.068.

Deng, Yu; Zhang, Duo-rui; Xia, Jin-Lan; Nie, Zhen-Yuan; Liu, Hong-Chang; Wang, Na; Xue, Zhen (2020b): Enhancement of arsenopyrite bioleaching by different Fe(III) compounds through changing composition and structure of passivation layer. In *Journal of Materials Research and Technology* 9 (6), pp. 12364–12377. DOI: 10.1016/j.jmrt.2020.08.088.

Deveci, H.; Akcil, A.; Alp, I. (2004): Bioleaching of complex zinc sulphides using mesophilic and thermophilic bacteria: comparative importance of pH and iron. In *Hydrometallurgy* 73 (3-4), pp. 293–303. DOI: 10.1016/j.hydromet.2003.12.001.

Dopson, Mark; Lövgren, Lars; Boström, Dan (2009): Silicate mineral dissolution in the presence of acidophilic microorganisms: Implications for heap bioleaching. In *Hydrometallurgy* 96 (4), pp. 288–293. DOI: 10.1016/j.hydromet.2008.11.004.

Drewniak, L.; Sklodowska, A. (2013): Arsenic-transforming microbes and their role in biomining processes. In *Environmental science and pollution research international* 20 (11), pp. 7728–7739. DOI: 10.1007/s11356-012-1449-0.

Fowler, T. A.; Crundwell, F. K. (1999): Leaching of zinc sulfide by Thiobacillus ferrooxidans: bacterial oxidation of the sulfur product layer increases the rate of zinc sulfide dissolution at high concentrations of ferrous ions. In *Applied and environmental microbiology* 65 (12), pp. 5285–5292. DOI: 10.1128/AEM.65.12.5285-5292.1999.

Fu, Kai-bin; Lin, Hai; Mo, Xiao-lan; Wang, Han; Wen, Hong-wei; Wen, Zi-long (2012): Comparative study on the passivation layers of copper sulphide minerals during bioleaching. In *Int J Miner Metall Mater* 19 (10), pp. 886–892. DOI: 10.1007/s12613-012-0643-x.

Gunn, Gus (2014): Critical metals handbook. Hoboken NJ: John Wiley & Sons.

Irrgang, Nico; Monneron-Enaud, Benjamin; Möckel, Robert; Schlömann, Michael; Höck, Michael (2021): Economic feasibility of the co-production of indium from zinc sulphide using bioleaching extraction in Germany. In *Hydrometallurgy* 200 (7), p. 105566. DOI: 10.1016/j.hydromet.2021.105566.

Jia Feng Li; Lin Lin Tong; Qiao Chen; Zhe Nan Jin; Hong Ying Yang: Bioleaching Experiments on a Low-Grade Complex Zinc Ore from Inner Mongolia.

Jiang, Jibo; Liang, Duoqiang; Zhong, Qingdong (2011): Precipitation of indium using sodium tripolyphosphate. In *Hydrometallurgy* 106 (3-4), pp. 165–169. DOI: 10.1016/j.hydromet.2010.12.009.

Jowkar, Mohammad Javad; Bahaloo-Horeh, Nazanin; Mousavi, Seyyed Mohammad; Pourhossein, Fatemeh (2018): Bioleaching of indium from discarded liquid crystal displays. In *Journal of Cleaner Production* 180, pp. 417–429. DOI: 10.1016/j.jclepro.2018.01.136.

Justice, Nicholas B.; Norman, Anders; Brown, Christopher T.; Singh, Andrea; Thomas, Brian C.; Banfield, Jillian F. (2014): Comparison of environmental and isolate Sulfobacillus genomes reveals diverse carbon, sulfur, nitrogen, and hydrogen metabolisms. In *BMC genomics* 15, p. 1107. DOI: 10.1186/1471-2164-15-1107.

Keeling, S. E.; Palmer, M-L.; Caracatsanis, F. C.; Johnson, J. A.; Watling, H. R. (2005): Leaching of chalcopyrite and sphalerite using bacteria enriched from a spent chalcocite heap. In *Minerals Engineering* 18 (13-14), pp. 1289–1296. DOI: 10.1016/j.mineng.2005.05.015.

Krause, E.; Ettel, V. A. (1989): Solubilities and stabilities of ferric arsenate compounds. In *Hydrometallurgy* 22 (3), pp. 311–337. DOI: 10.1016/0304-386X(89)90028-5.

Liu; Li, Qian; Zhang; Yang; Xu; Jiang (2019): Formation Process of the Passivating Products from Arsenopyrite Bioleaching by Acidithiobacillus ferrooxidans in 9K Culture Medium. In *Metals* 9, p. 1320. DOI: 10.3390/met9121320.

Liu, Ronghui; Zhou, Hongbo (2022): Growth in ever-increasing acidity condition enhanced the adaptation and bioleaching ability of Leptospirillum ferriphilum. In *International microbiology : the official journal of the Spanish Society for Microbiology* 25 (3), pp. 541–550. DOI: 10.1007/s10123-021-00227-4.

Lotfalian, M.; Ranjbar, M.; Fazaelipoor, M. H.; Schaffie, M.; Manafi, Z. (2015): The effect of redox control on the continuous bioleaching of chalcopyrite concentrate. In *Minerals Engineering* 81, pp. 52–57. DOI: 10.1016/j.mineng.2015.07.006.

Martin, Mirko; Janneck, Eberhard; Kermer, René; Patzig, Anke; Reichel, Susan (2015): Recovery of indium from sphalerite ore and flotation tailings by bioleaching and subsequent precipitation processes. In *Minerals Engineering* 75 (11), pp. 94–99. DOI: 10.1016/j.mineng.2014.11.015.

Martin Lokanc, Roderick Eggert, and Michael Redlinger: Colorado School of Mines, Golden, Colorado: The Availability of Indium: The Present, Medium Term, and Long Term.(2015)

Mishra, Debaraj; Kim, Dong-Jin; Ahn, Jong-Gwan; Rhee, Young-Ha (2005): Bioleaching: A microbial process of metal recovery; A review. In *Met. Mater. Int.* 11 (3), pp. 249–256. DOI: 10.1007/BF03027450.

Moss R, Tzimas E, Kara H, Willis P, Kooroshy J.: Critical Metals in Strategic Energy Technologies - Assessing Rare Metals as Supply-Chain Bottlenecks in Low-Carbon Energy Technologies.(2011) Available online at https://publications.jrc.ec.europa.eu/repository/handle/JRC65592.

Ngoma, Emmanuel; Borja, Danilo; Smart, Mariette; Shaik, Kathija; Kim, Hyunjung; Petersen, Jochen; Harrison, Susan T.L. (2018): Bioleaching of arsenopyrite from Janggun mine tailings (South Korea) using an adapted mixed mesophilic culture. In *Hydrometallurgy* 181, pp. 21–28. DOI: 10.1016/j.hydromet.2018.08.010.

Pourhossein, Fatemeh; Rezaei, Omid; Mousavi, Seyyed Mohammad; Beolchini, Francesca (2021): Bioleaching of critical metals from waste OLED touch screens using adapted acidophilic bacteria. In *Journal of environmental health science & engineering* 19 (1), pp. 893–906. DOI: 10.1007/s40201-021-00657-2.

Rathna, Ravichandran; Nakkeeran, Ekambaram (2020): Chapter 22 - Biological treatment for the recovery of minerals from low-grade ores. In Sunita Varjani, Ashok Pandey, Edgard Gnansounou, Samir Kumar Khanal, Sindhu Raveendran (Eds.): Current Developments in Biotechnology and Bioengineering: Elsevier, pp. 437–458. Available online at https://www.sciencedirect.com/science/article/pii/B9780444643216000227.

Schippers, Axel; Sand, Wolfgang (1999): Bacterial Leaching of Metal Sulfides Proceeds by Two Indirect Mechanisms via Thiosulfate or via Polysulfides and Sulfur. In *Applied and environmental microbiology* 65, pp. 319–321. DOI: 10.1128/AEM.65.1.319-321.1999.

Seifert, Thomas; Sandmann, Dirk (2006): Mineralogy and geochemistry of indium-bearing polymetallic vein-type deposits: Implications for host minerals from the Freiberg district, Eastern Erzgebirge, Germany. In *Ore Geology Reviews* 28 (1), pp. 1–31. DOI: 10.1016/j.oregeorev.2005.04.005.

Ulrich Schwarz-Schampera; Peter M. Herzig (2010): Indium: Geology, Mineralogy, and Economics. In.

Vera, Mario; Schippers, Axel; Sand, Wolfgang (2013): Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation--part A. In *Applied microbiology and biotechnology* 97 (17), pp. 7529–7541. DOI: 10.1007/s00253-013-4954-2.

Vilcáez, J.; Suto, K.; Inoue, C. (2008): Bioleaching of chalcopyrite with thermophiles: Temperature–pH–ORP dependence. In *International Journal of Mineral Processing* 88 (1-2), pp. 37–44. DOI: 10.1016/j.minpro.2008.06.002.

Violante, Antonio; Del Gaudio, Stefania; Pigna, Massimo; Ricciardella, Mariarosaria; Banerjee, Dipanjan (2007): Coprecipitation of Arsenate with Metal Oxides. 2. Nature, Mineralogy, and Reactivity of Iron(III) Precipitates. In *Environmental Science & Technology* 41 (24), pp. 8275–8280. DOI: 10.1021/es070382+.

Wanjiya, Mwema; Chowdhury, Faysol; Ojumu, Tunde Victor (2015): Solution pH and Jarosite Management during Ferrous Iron Biooxidation in a Novel Packed-Column Bioreactor. In *AMR* 1130, pp. 291–295. DOI: 10.4028/www.scientific.net/AMR.1130.291.

Watling, H. R.; Collinson, D. M.; Li, J.; Mutch, L. A.; Perrot, F. A.; Rea, S. M. et al. (2014): Bioleaching of a low-grade copper ore, linking leach chemistry and microbiology. In *Minerals Engineering* 56, pp. 35–44. DOI: 10.1016/j.mineng.2013.10.023.

Wood, Scott A.; Samson, Iain M. (2006): The aqueous geochemistry of gallium, germanium, indium and scandium. In *Ore Geology Reviews* 28 (1), pp. 57–102. DOI: 10.1016/j.oregeorev.2003.06.002.

Yin, Lu; Yang, Hong-Ying; Tong, Lin-Lin; Ma, Peng-Cheng; Zhang, Qin; Zhao, Miao-Miao (2020): Arsenopyrite Bio-Oxidization Behavior in Bioleaching Process: Evidence From Laser Microscopy, SEM-EDS, and XPS. In *Frontiers in microbiology* 11, p. 1773. DOI: 10.3389/fmicb.2020.01773.