IRSTI 34.35.25 https://doi.org/10.26577/EJE208830626

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RECHAR-M2C: MICROBIAL ISOLATION FROM LEGACY-CONTAMINATED SOILS

The present study aimed to isolate Zn-solubilising and metal-tolerant microbial strains from legacy-contaminated soils affected by potentially toxic elements (PTEs), persistent organic pollutants (POPs), or their combination. A total of 242 microbial strains were isolated. Subsequent sequential screening for Zn-solubilisation potential identified 13 isolates with solubilisation indices exceeding 1.5. Of these, four isolates (S21, S56, TA5, and TE32) exhibited strong tolerance to 0.03–0.06% Zn, with relative growth intensities (RGIs) ranging from 86.6% to 104%, while S56 and TE32 maintained sufficient tolerance at a Zn concentration of 0.12%. Regarding Ni tolerance, only TE32 exhibited sufficient resistance at a Ni concentration of 0.03%, with an RGI of 47.2%. For Cu tolerance, isolates S21, S56, and TE32 demonstrated strong resistance at a Cu concentration of 0.03%, with RGIs of 92.8%, 82.4%, and 79.6%, respectively. Furthermore, TE32 maintained sufficient tolerance at 0.06% Cu, with an RGI of 63.0%. Based on these results, three isolates (S21, S56, and TE32) were selected for comprehensive characterisation of their enzymatic (amylolytic, cellulolytic, lipolytic, and proteolytic) and plant growth-promoting traits, including siderophore, extracellular polymeric saccharide (EPS), and indole-3-acetic acid (IAA) production. Strain S21 exhibited moderate proteolytic activity, siderophore production, and superior IAA synthesis, reaching a peak concentration of 38.7 ± 3.29 μg mL^{-1}. Strain S56 demonstrated moderate proteolytic activity, strong lipolytic and proteolytic activities, siderophore production, and moderate IAA synthesis, with a maximum concentration of 17.5 ± 1.48 μg mL^{-1}. In contrast, strain TE32 exhibited strong amylolytic activity, proteolytic activity, EPS production, and the lowest IAA-producing capacity, reaching a peak concentration of 12.8 ± 0.88 μg mL^{-1}. Future research will focus on the development of microbe–biochar complexes through the immobilisation of microbial cells onto biochar surfaces, followed by validation of the developed complexes in marginal and/or contaminated soils to enhance plant growth.

Keywords: legacy soil contamination, potentially toxic elements, Zn-solubilising microorganisms, tolerance to heavy metals, plant growth-promoting microorganisms.

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Rechar-M2C: тарихи ластанған топырақтардан микроорганизмдерді бөліп алу

Осы зерттеудің мақсаты потенциалды уытты элементтердің (ПУЭ), тұрақты органикалық ластағыштардың (ТОЛ) немесе олардың комбинациясының әсеріне ұшыраған тарихи ластанған топырақтардан Zn ерітуге қабілетті және металдарға төзімді микробтық штамдарды бөліп алу болды. Барлығы 242 микробтық штамм бөлініп алынды. Кейінгі дәйекті скрининг нәтижесінде Zn солюбилизациялау әлеуеті бойынша солюбилизация индекстері 1,5-тен жоғары 13 изолят анықталды. Олардың ішінде төрт изолят (S21, S56, TA5 және TE32) 0,03–0,06% Zn жағдайында жоғары төзімділік көрсетті, салыстырмалы өсу қарқындылығы (RGI) 86,6%-дан 104%-ға дейін өзгерді, ал S56 және TE32 изоляттары Zn концентрациясы 0,12% болған жағдайда да жеткілікті төзімділікті сақтады. Ni-ге төзімділікке қатысты, тек TE32 изоляты Ni концентрациясы 0,03%

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болғанда жеткілікті төзімділік көрсетті, оның RGI мәні 47,2% құрады. Cu-ге төзімділік бойынша S21, S56 және TE32 изоляттары Cu концентрациясы 0,03% болғанда жоғары төзімділік танытты, олардың RGI мәндері тиісінше 92,8%, 82,4% және 79,6% болды. Сонымен қатар, TE32 изоляты 0,06% Cu жағдайында да жеткілікті төзімділікті сақтап, RGI мәні 63,0% құрады. Осы нәтижелер негізінде үш изолят (S21, S56 және TE32) олардың ферментативтік қасиеттерін (амилолитикалық, целлюлолитикалық, липолитикалық және протеолитикалық белсенділіктерін), сондай-ақ өсімдік өсуін ынталандыратын белгілерін, соның ішінде сидерофорлар, жасушадан тыс полимерлік сахаридтер (EPS) және индол-3-сірке қышқылы (ИСҚ) өндіру қабілетін кешенді сипаттау үшін таңдап алынды. S21 штаммы орташа протеолитикалық белсенділік, сидерофорлар өндіру қабілеті және ИСҚ синтезінің ең жоғары деңгейін көрсетті, оның ең жоғары концентрациясы 38,7 ± 3,29 мкг/мл болды. S56 штаммы орташа протеолитикалық белсенділік, күшті липолитикалық және протеолитикалық белсенділіктер, сидерофорлар өндіру қабілеті және орташа ИСҚ синтезін көрсетті, ең жоғары концентрациясы 17,5 ± 1,48 мкг/мл құрады. Ал TE32 штаммы күшті амилолитикалық белсенділік, протеолитикалық белсенділік, EPS өндіру қабілеті және ИСҚ өндірудің ең төмен әлеуетін көрсетті, оның ең жоғары концентрациясы 12,8 ± 0,88 мкг/мл болды. Болашақ зерттеулер микробтық жасушаларды биокөмір бетіне иммобилизациялау арқылы микроб–биокөмір кешендерін әзірлеуге, кейін әзірленген кешендерді өсімдіктердің өсуін күшейту мақсатында өнімділігі төмен және/немесе ластанған топырақтарда валидациялауға бағытталатын болады.

Түйін сөздер: тарихи ластанған топырақ, потенциалды уытты элементтер, Zn еріту қабілеті бар микроорганизмдер, ауыр металдарға төзімділік, өсімдік өсуін ынталандыратын микроорганизмдер.

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Rechar-M2c: выделение микроорганизмов из почв с историческим загрязнением

Целью данного исследования было выделение из почв с историческим загрязнением, подвергшихся воздействию потенциально токсичных элементов (ПТЭ), стойких органических загрязнителей (СОЗ) или их сочетания, микробных штаммов, способных солюбилизировать Zn и обладающих толерантностью к металлам. Всего было выделено 242 микробных штамма. Последующий последовательный скрининг на Zn-солюбилизирующую активность позволил выявить 13 изолятов с индексами солюбилизации выше 1,5. Среди них четыре изолята (S21, S56, TA5 и TE32) проявили высокую толерантность к 0,03–0,06% Zn, при этом относительная интенсивность роста (RGI) варьировала от 86,6% до 104%, тогда как S56 и TE32 сохраняли достаточную толерантность при концентрации Zn 0,12%. Что касается толерантности к Ni, только TE32 проявил достаточную устойчивость при концентрации Ni 0,03%, с RGI 47,2%. В отношении толерантности к Cu изоляты S21, S56 и TE32 продемонстрировали высокую устойчивость при концентрации Cu 0,03%, с RGI 92,8%, 82,4% и 79,6% соответственно. Кроме того, TE32 сохранял достаточную толерантность при 0,06% Cu, с RGI 63,0%. На основании полученных результатов три изолята (S21, S56 и TE32) были отобраны для комплексной характеристики их ферментативных свойств (амилолитической, целлюлолитической, липолитической и протеолитической активности), а также признаков, способствующих росту растений, включая продукцию сидерофоров, внеклеточных полимерных сахаридов (ЭПС) и индол-3-уксусной кислоты (ИУК). Штамм S21 проявил умеренную протеолитическую активность, способность к продукции сидерофоров и наиболее выраженный синтез ИУК, достигая максимальной концентрации 38,7 ± 3,29 мкг/мл. Штамм S56 продемонстрировал умеренную протеолитическую активность, выраженную липолитическую и протеолитическую активности, продукцию сидерофоров и умеренный синтез ИУК, с максимальной концентрацией 17,5 ± 1,48 мкг/мл. В отличие от них, штамм TE32 проявил выраженную амилолитическую активность, протеолитическую активность, продукцию ЭПС и наименьшую способность к продукции ИУК, достигая максимальной концентрации 12,8 ± 0,88 мкг/мл. Дальнейшие исследования будут направлены на разработку микробно-биочарных комплексов посредством иммобилизации микробных клеток на поверхности биочара с последующей валидацией разработанных комплексов в деградированных и/или загрязнённых почвах для оптимизации развития растений.

Ключевые слова: историческое загрязнение почв, потенциально токсичные элементы, Zn-солюбилизирующие микроорганизмы, толерантность к тяжёлым металлам, микроорганизмы, способствующие росту растений.

Introduction

Extensive research is currently focused on the restoration of degraded, marginal, and contaminated lands in response to the United Nations report on the status of the Sustainable Development Goals (SDGs). As of 14 June 2025, SDG target 15.3, which aims to restore degraded lands, was classified as showing regression (UN, 2025), highlighting the urgent need to develop effective and sustainable solutions capable of reversing this negative trend and, at a minimum, preventing further deterioration.

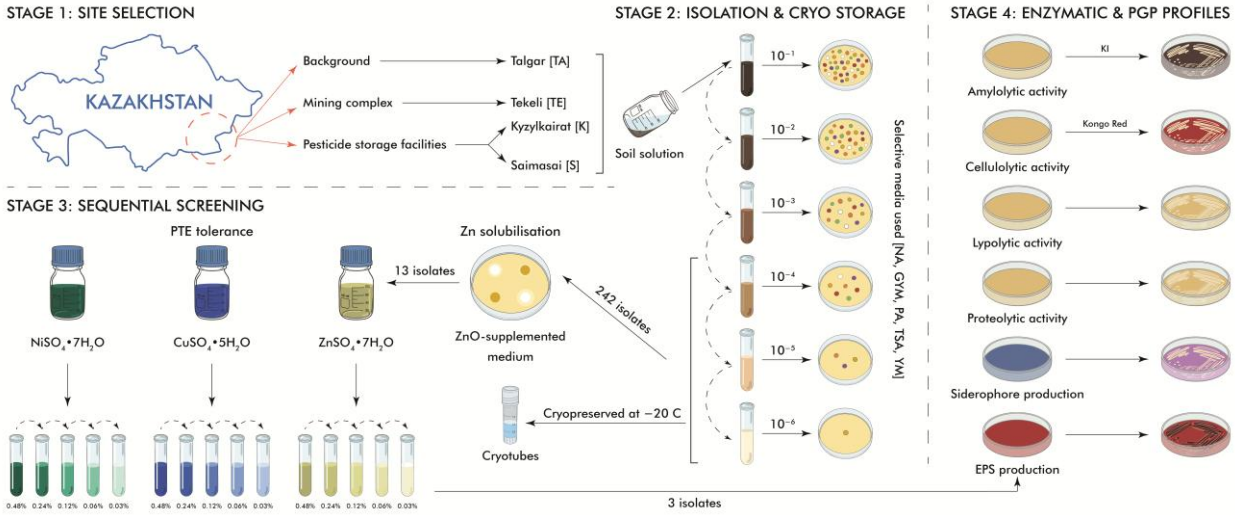
These challenges coincide with the broader evolution of resource management concepts. Since the introduction of the term "value chain" by Porter (1985), originally describing the creation of value-added products through continuous reintegration into biological or technical cycles (D'heur, 2015), the concept has been increasingly adopted within scientific research (Althuri & Venkata Mohan, 2022; Aso, 2020; Carvalho et al., 2021; Casarejos et al., 2018). In parallel, the concept of the bioeconomy, introduced by the European Commission in 2012, promoted resource recovery and reuse in place of dependence on external raw materials (European Commission & Directorate-General for Research and Innovation, 2012). These developments have accelerated the transition from a linear to a circular economic model (Khaw-ngern et al., 2021; Corona et al., 2019; European Commission et al., 2021; Khaw-ngern et al., 2021; Wiesmeth, 2021). Simultaneously, phytoremediation, as one of the most promising sustainable and economically feasible technology, has progressively evolved into the broader concept of phytomanagement (Evangelou et al., 2015; Stephenson & Black, 2014), which integrates environmental restoration with the production of economically valuable biomass. Furthermore, during the past decade, the technology has evolved towards microbe-assisted and biochar-assisted phytoremediation (Nurzhanova et al., 2025, 2026; Pidlisnyuk et al., 2020, 2022; Pidlisnyuk, Newton, et al., 2025). Despite its considerable potential for restoring contaminated soils, increasing concern has been raised regarding the annual generation of post-phytoremediation biomass, often referred to as "waste" or "unconditioned" biomass. The need for careful management of this biomass was first emphasised by Song & Park (2017), who called for the development of policies addressing "post-phytoremediation management". This was subsequently reinforced by numerous studies highlighting the necessity of implementing sustainable post-harvest management strategies (A.H. A. Khan et al., 2023; Priya et al., 2023; Tan et al., 2023). Such concerns are particularly relevant because biomass harvested from potentially toxic element (PTE)-contaminated sites may contain elevated concentrations of PTEs (Pidlisnyuk, Mamirova, et al., 2025).

Therefore, it has been proposed that post-phytoremediation biomass could instead be valorised through conversion into biochar and subsequently reused on contaminated soils to improve soil health and promote plant development (Pidlisnyuk, Mamirova, et al., 2025). Although numerous studies have demonstrated that biochar application improves soil fertility, enhances nutrient availability, stimulates microbial abundance, and promotes plant growth (Fox et al., 2016; Houben et al., 2014; W.-D. Khan et al., 2017; Mierzwa-Hersztek et al., 2017), several important limitations remain (Brtnicky et al., 2021; Ding et al., 2016). Among these, the most critical is the desorption of toxic substances, particularly PTEs, which may result in secondary contamination (2C) (Ding et al., 2016; George, 2022). Consequently, when post-phytoremediation biomass is converted into biochar, the potential risk of 2C must be carefully considered. Recent studies have demonstrated that Miscanthus-derived biochar may contain elevated concentrations of Mn, Zn, and Sr (Kononchuk et al., 2022; Pidlisnyuk, Newton, et al., 2025). Although Zn is an essential micronutrient for plant growth, excessive concentrations induce phytotoxicity, forcing plants to divert resources from development towards defence responses and activation of avoidance strategies (Rai et al., 2019; Robb, 2013). Consequently, the incorporation of Zn-enriched waste-derived biochar into soil may create a substantial risk of 2C, potentially reducing biomass productivity and impairing the phytoremediation process. Indeed, recent evidence has demonstrated that biochar produced from waste biomass may release Cu and Zn into soil, thereby creating an additional environmental concern (Nurzhanova et al., 2025). Mitigation of this risk therefore requires the development of effective biochar conditioning strategies before its application. A review of the available literature revealed remarkably limited research addressing the prevention of 2C associated with contaminated biochar. One of the few reported approaches involved loading nano zero-valent iron

onto biochar followed by re-pyrolysis to stabilise Cr and reduce its bioavailability (Fan et al., 2019).

To address this knowledge gap, the project "ReChar-M2C", officially entitled AP23490702 "Development of Biochar Decontamination Technology for Sustainable Waste Biomass Conversion into Value-Added Products", was initiated to develop a microbial biochar reconditioning technology aimed at preventing secondary contamination through biochar decontamination. The proposed approach is based on reducing the metal or PTE load in biochar by exploiting the metal-leaching capacity of selected microbial isolates. The present study represents the first stage of this project and focuses on the isolation of Zn-solubilising and PTE-tolerant microorganisms from legacy-contaminated soils. Such environments were selected as potential reservoirs of microorganisms possessing multiple desirable traits and capable of serving as biological agents for biochar de-metalisation (Figure 1).

Figure 1
Experimental workflow visualisation



Materials and methods

Soil Characterisation

Site Description

Four sites (Table 1) located in the Almaty and Zhetysu regions (Kazakhstan) were selected for microbial isolation based on previously reported contamination (Baubekova et al., 2021; Mamirova et al., 2022; Nurzhanova et al., 2025). At each site, an area of 25 m^2 was designated for sampling.

According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2022), soils from Sites 1–3 and Site 4 were classified as Chernozem and Kastanozems, respectively. Based on Köppen–Geiger classification (Beck et al., 2018; Kottek et al., 2006), the climate of Sites 1–4 was classified as *Dfa* (snow–fully humid–hot summer) in 2006 and *BSk* (arid–steppe–cold) in 2016.

Soil Collection

Soil sampling adhered to the ISO 18400–203:2018 (ISO 18400-203:2018, 2018) and ISO 18400–206:2018 (ISO 18400-206:2018, 2018) for chemical and microbiological analyses, respectively. Five samples were procured from investigated areas, with each sample taken from a 5 × 5 m^2 area at a depth of 0–0.6 m. Following sampling, collected soil was sieved (d = 3 mm) to remove the plant materials and stones, thoroughly homogenized, air-dried, and stored at 4 °C before passing for agrochemical and chemical analyses.

Table 1
Site description

Site		1	2	3	4
Designated name		TA	K	S	TE
Type of soil		Background	Complexly contaminated		PTE-contaminated
Sampling location	Territory	foot of Talgar Peak	–	–	–
	Village	–	Kyzylkairat	Saimasai	–
	City	Talgar	–	–	Tekeli
Region		Almaty			Zhetysu
GPS coordinates		43°16'36.0" N, 77°12'37.0" E	43°17'58.7" N, 77°11'39.6" E	43°27'19.5" N, 77°18'51.5" E	44°53'15" N, 78°37'34" E
Contamination	PTEs	✓	✓	✓	✓
	POPs	✗	✓	✓	✗
Contamination origin		✗	Former pesticide storage facility		Mining complex

Agrochemical Analysis

Agrochemical parameters of the research soils 1–4 were determined using standard methods: total organic content (TOC) – by Tyurin's method (ST RK 3477-2019, 2019); available phosphorus (P_2O_5) and potassium (K_2O) – by Machigin method in CRIAAS modification (GOST 26205-91, 1993); the absorbed bases of sodium (Na) and potassium (K) – by Antipov-Karataev and Mametov method in Grabarov modification; the absorbed bases of calcium (Ca) and magnesium (Mg) – by Arinushkin method in Grabarov modification; and the soil pH – in accordance with the GOST 26423-85 (GOST 26423-85, 2011). The agrochemical profiles of research soils are presented in Table 2.

Table 2
Agrochemical profiles of studied soils

Parameter	Unit	TA soil	K soil	S soil	TE soil	*p*-value
pH (H_2O)	–	7.79 ± 0.04 **a**	7.44 ± 0.03 **c**	7.64 ± 0.02 **b**	6.68 ± 0.06 **d**	< 0.001
TOC	%	1.23 ± 0.08 **c**	2.93 ± 0.02 **b**	2.81 ± 0.07 **b**	4.31 ± 0.03 **a**	< 0.001
P_2O_5	mg kg^{-1}	9.00 ± 1.00 **c**	19.0 ± 1.00 **b**	61.0 ± 1.00 **a**	21.0 ± 1.00 **b**	< 0.001
K_2O		115 ± 5.00 **d**	200 ± 0 **b**	340 ± 0 **a**	180 ± 0 **c**	< 0.001
NO_3		0.59 ± 0.02 **b**	1.66 ± 0.05 **a**	0.60 ± 0.05 **b**	0.58 ± 0.05 **b**	< 0.001
NH_4		1.35 ± 0.25 **c**	3.70 ± 0.60 **b**	1.50 ± 0.20 **c**	4.90 ± 0.50 **a**	< 0.001
Ca	mg Eq/100 g	13.3 ± 0.24 **c**	16.3 ± 0.73 **b**	10.7 ± 0 **d**	21.1 ± 0.24 **a**	< 0.001
Mg		1.70 ± 0.24 **b**	3.40 ± 0.97 **b**	13.8 ± 1.68 **a**	3.16 ± 0.25 **b**	< 0.001
Na		0.15 ± 0 **a**	0.15 ± 0 **a**	0.08 ± 0 **b**	0.15 ± 0 **a**	< 0.001
K		0.06 ± 0 **a**	0.06 ± 0 **a**	0.04 ± 0 **b**	0.06 ± 0 **a**	< 0.001

Notes: TA – Talgar; K – Kyzylkairat; S – Saimasai; TE – Tekeli; TOC – total organic carbon.

Determination of Potentially Toxic Elements Content

The determination of 8 elements (Cr, Co, Ni, Cu, Zn, As, Cd, and Pb) in soil samples was conducted using a mass spectrometer with inductively coupled plasma (ICP-MS) Agilent 7500 series (Agilent Technologies, Inc., USA) following ST RK ISO 11047–2008 (ST RK ISO 11047-2008, 2009) and GD 52.18.721-2009 (GD 52.18.721-2009, 2010). The decomposition of soil samples was performed in a microwave sample decomposition system Speedwave Four, Berghof, following the Soil EPA 3051A (US EPA 3051A, 2007) standard. For this purpose, a sample of soil weighing (1.0 ± 0.5 g) with

an accuracy of 4 digits was utilized. The samples were placed in a Teflon autoclave, to which 6.0 mL of concentrated nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) were added. The Teflon autoclave was sealed and then put in a SpeedWave Four microwave digestion system. Afterwards, the samples were cooled to room temperature. The solution with the precipitate was transferred to a volumetric flask and brought to the 25.0 mL mark with bi-distilled water (dH_2O). The sample was filtered and subsequently analysed on a mass spectrometer.

In order to evaluate soil contamination levels in relation to PTEs, geo-accumulation index (I_{geo}), soil toxicity index (Z_{ci}), ecological risk factor (*ER*), and potential ecological risk index (*PERI*) were calculated.

Geo-accumulation index (I_{geo}) was used as common index for the assessment of soil pollution with PTEs (Saleem et al., 2024) and calculated using the following equation:

$$I_{geo} = log_2\left(\frac{C_i}{1.5 \times C_{g\varphi i}}\right) \quad (1)$$

where I_{geo} – geo-accumulation index; C_i – actual concentration of *i* element in soil (mg kg^{-1}); $C_{g\phi i}$ – geochemical background concentration of *i* element (mg kg^{-1}; Table 3) (Lide, 2005).

Soil toxicity index (Z_{ci}) was used as comprehensive method representing cumulative soil contamination (Vodyanitskii, 2010) and calculated as follows:

$$Z_{ci} = \Sigma(K_{Ci} \times K_{Ti} + \cdots + K_{cn} \times K_{Tn}) - (n-1) \quad (2)$$

$$K_C = C_i / C_{\varphi i} \quad (3)$$

where K_C – concentration coefficient of *i* element; K_T – toxicity coefficient of *i* element (Table 3); *n* – number of elements with K_c >1; C_i – actual concentration of *i* element (mg kg^{-1}); $C_{\phi i}$ – maximum permissible concentration of *i* element (mg kg^{-1}).

Ecological risk factor (E_R) was used as indicator of ecological risk for each PTE separately (Hakanson, 1980) and calculated as follows:

$$E_R = T_R \times C_{Fi} \quad (4)$$

$$C_{Fi} = C_i / C_{g\varphi i} \quad (5)$$

where T_R – toxic response factor of element (Table 3) (Hakanson, 1980; Siddig et al., 2025); C_{Fi} – contamination factor of element; C_i – actual concentration of *i* element in soil (mg kg^{-1}); $C_{g\phi i}$ – geochemical background concentration of *i* element (mg kg^{-1}; Table 3) (Lide, 2005).

Potential ecological risk index (PERI) was used as a comprehensive method representing cumulative toxicological effect and calculated as follows (Hakanson, 1980):

$$PERI = \sum_{i=1}^{n} E_R \quad (6)$$

Table 3
Values and scales used for estimation of soil contamination levels

PTE	MPC*	$C_{g\phi i}$	K_T	T_R	Scale	I_{geo}	Z_{ci}	E_R	*PERI*
Cr	90	102	1.5	2.00	Safe/low	< 1	< 16	< 40	< 150
Co	5.0	25.0	1.0	5.00	Moderate	> 1 < 3	> 16 < 32	> 40 < 80	> 150 < 300
Ni	20	84.0	1.5	5.00					
Cu	33	60.0	1.0	5.00	Considerable	–	> 32 < 128	> 80 < 160	> 300 < 600
Zn	55	70.0	1.5	1.00					
Cd	0.5	0.15	1.5	30.0	Heavy	> 3 < 5	–	> 160 < 320	–
As	2.0	1.80	1.5	10.0					
Pb	32 [a]	14.0	1.5	5.00	Extreme	> 5	> 128	> 320	> 600

*Notes: * – maximum permissible concentrations for mobile fractions of elements (mg kg^{-1}) (MH RK, 2021); [a] – MPC for total concentration; $C_{g\phi i}$ – geochemical background concentration of i element (mg kg^{-1}) (Lide, 2005); K_T – toxicity coefficient of i element (Vodyanitskii, 2010); T_R – toxic response factor of element (Hakanson, 1980; Siddig et al., 2025); I_{geo} – geo-accumulation index, contamination (Saleem et al., 2024); Z_{ci} – soil toxicity index, danger (Vyborov et al., 2004); E_R – ecological risk factor, risk (Hakanson, 1980); PERI – potential ecological risk index, risk (Hakanson, 1980).*

Determination of Persistent Organic Pollutants Content

POP-pesticides concentrations, including 21 compounds, were measured in studied soils in accordance with ST RK 2131-2011 (ST RK 2131-2011, 2012). The analysed compounds included DDT metabolites (2.4′-DDD, 4.4′-DDD, 4.4′-DDE, and 4.4′-DDT), HCH isomers (α−, β−, γ−, and δ−), aldrin, chlordane, dieldrin, dicofol, endosulfan (α and β), endosulfan sulfate, endrin, endrin aldehyde, hexachlorobenzene (HCB), heptachlor, heptachlor epoxide, and methoxychlor. Analyses were performed using a Gas Chromatograph 6890N with an Electron Capture Detector (Agilent Technologies, Inc., USA) equipped with a Combi-PAL autosampler (CTC Analytics AG, Switzerland).

To extract POPs, 10 g of the sample was placed in a stopper 200 mL flask, 20 mL of n-hexane was added, and the mixture was shaken for 24 h. The volume of the obtained extract was measured and concentrated to 1 mL. The extract was placed in a 50-mL conical flask, 5-10 mL of *n*-hexane was added, and the flask was stirred for 30 min in a mixer. The resulting extract was filtered through filter paper, measured, and concentrated to 1 mL. 1 μL volume of the finalized extract was injected using an autosampler for introducing a sample to be heated to 250°C. For separation, a capillary column DB−35MS (Agilent Technologies, Inc., USA) with a length of 30 m, internal diameter of 0.25 mm, and 0.25 mm thick film was used. The carrier gas (helium mark "A") was fed with a continuous flow rate of 1.0 mL min^{-1} (corresponding to an average linear flow velocity of 36 cm s^{-1}). The temperature of the thermostat column was programmed to heat up from 40°C (hold time 1 min) to 160°C (hold time 3 min) at a heating rate of 20°C min^{-1}, followed by further heating to 250°C (hold time 5 min) at 3°C min^{-1}. Detection was conducted by an electron capture device. For quantitative determination of POPs, calibration plots were obtained over a concentration range of 1–500 ng L^{-1}. The chromatography results were processed using MSD ChemStation software E.02.02 SP1.

Microbial Isolation & Cryopreservation

Upon arrival at the laboratory, the collected soil samples were air-dried at room temperature, sieved (d = 2 mm), and processed according to the procedure described by McPherson et al. (McPherson et al., 2018). Microorganisms were isolated using a serial dilution technique on different agar media (Janati et al., 2022). The sieved soil (10 g) was suspended in 90 mL sterile phosphate-buffered saline (PBS, pH 7.2) and shaken at 150 rpm for 2 h. The resulting suspension was subsequently diluted by transferring 1 mL into 9 mL of 0.1% peptone water to obtain serial dilutions ranging from 10^{-1} to 10^{-6}. Aliquots (0.1 mL) of each dilution were plated onto Nutrient agar (NA), Tryptic soy agar (TSA), Pseudomonas agar (PA), Glucose yeast malt agar (GYM), and Yeast malt agar (YM) media (Table 4).

Inoculated plates were incubated at 28–30 °C for 48 h. Morphologically distinct colonies were selected and purified by repeated subculturing (Madigan et al., 2018). Following the establishment of pure cultures, all isolates were characterised morphologically, including colony colour, margins, surface texture, shape, elevation, and pigmentation (Mohite, 2013). All isolates were cryopreserved and stored at −20 °C. In addition, all microbial cultures were screened for growth on NA medium.

Screening for Zn-Solubilising Activity

Zn-solubilising microorganisms were screened on Zn-supplemented medium with the following composition (g L^{-1}): 0.1 K_2HPO_4; 1.0 $(NH_4)_2SO_4$; 0.2 KCl; 0.2 $MgSO_4 \cdot \bullet 7H_2O$; 10 dextrose; 15.0 agar; and 1.245 ZnO. Colonies aged 24–48 h were transferred into the corresponding broth media (NA, GYM, or YM) and incubated at 28 °C and 160 rpm for 16 h. Following incubation, 10 μL of the microbial suspension was spot-inoculated onto the surface of control (without Zn) and 0.1% Zn-supplemented media. I Inoculations were performed in triplicate. The Petri plates were incubated in a thermostat at 28 °C for 7 days. After incubation, the diameters of the colonies and the clear ZnO dissolution zones were measured, and the solubilisation index (SI) was calculated according to Eq. 7 (Alexander & Zuberer, 1991; Boubekri et al., 2021):

$$SI = \frac{d_{Colony} + d_{Halo\ zone}}{d_{Colony}} \quad (7)$$

Table 4
Composition of agar media used for microbial isolation

Component	Unit	NA	TSA	PA	GYM	YM
Manufacturer	–	TM Media & HiMedia, India				
Premixed powder	g	15.0	30.0	45.03	–	–
pH (H_2O)		7.4 ± 0.2	7.3 ± 0.2	7.0 ± 0.2	7.2	6.2
Agar	g	20.0	20.0	13.6	20.0	20.0
$CaCO_3$	g	–	–	–	2.0	–
Glucose	g	–	2.5	–	4.0	10.0
Glycerol	g	–	–	20.0	–	–
Irgasan (Triclosan)	g	–	–	0.025	–	–
K_2HPO_4	g	–	2.5	–	–	–
K_2SO_4	g	–	–	10.0	–	–
Malt extract	g	–	–	–	10.0	3.0
Meat peptone	g	4.3	–	–	–	–
$MgCl_2$	g	–	–	1.40	–	–
NaCl	g	6.4	5.0	–	–	–
Peptone	g	–	–	20.0	–	5.0
Soya peptone	g	–	3.0	–	–	–
Tryptone	g	4.3	17.0	–	–	–
Yeast extract	g	–	–	–	4.0	3.0
dH_2O	mL	1,000	1,000	1,000	1,000	1,000

Notes: NA – nutrient agar; TSA – tryptic soy agar; PA – pseudomonas agar; GYM – glucose yeast malt agar; YM – yeast malt agar; dH_2O – distilled water.

Screening for PTE Tolerance

Screening of isolated microbial strains for tolerance to PTEs was conducted in two stages: (i) pre-screening for tolerance to 0.06% Zn to identify strains exhibiting superior resistance to elevated Zn concentrations; and (ii) comprehensive screening of the selected strains for tolerance to 0.03–0.48% Ni, Cu, and Zn to determine their resistance to PTEs commonly present in contaminated soils and to establish the range of concentrations tolerated.

For the pre-screening tolerance assay, the corresponding media described in Table 4 were used. To prepare Zn-supplemented media, 30 mL stock solutions containing 0.5% Zn were prepared using analytical-grade $ZnSO_4 \cdot 7H_2O$ (0.6596 g). The final Zn concentrations in the assay tubes were 0.06%.

For comprehensive tolerance assays, Müller–Hinton broth (MHB) was used, with the following composition (g L^{-1}): 2.0 beef extract, 17.5 acid hydrolysate of casein, and 1.5 starch; pH 7.3 ± 0.2. To prepare PTE-supplemented media, 30 mL metal stock solutions containing 3.52% of Ni, Cu, or Zn were prepared using analytical-grade salts: 4.6438 g of $ZnSO_4 \cdot 7H_2O$, 4.1493 g of $CuSO_4 \cdot 5H_2O$, and 5.0526 g of $NiSO_4 \cdot 7H_2O$. Serial two-fold dilution (15 mL stock solution + 15 mL dH_2O) was subsequently performed to obtain concentrations of 1.76%, 0.88%, 0.44%, and 0.22% for each metal. The final PTE concentrations in the assay tubes were 0.03%, 0.06%, 0.12%, 0.24%, and 0.48%.

The subsequent experimental procedures were identical for both screening stages. Specifically, a 24 h fresh culture of each strain was inoculated into 25 mL of the respective broth (MHB or the corresponding medium) and incubated at 28–30 °C and 140 rpm for 24 h. Following incubation, microbial suspensions were transferred to 2 mL cuvette, and optical density at 600 nm (OD_{600}) was measured using a SF-2000 spectrophotometer (OKB Spectr, Russian Federation), with uninoculated MHB or respective broth serving as the blank. This procedure ensured standardisation of the inoculum density, with OD_{600} of 0.010 ± 0.001 for both control and experimental tubes.

The experimental design included the following: blank (uninoculated respective broth), control 1 (inoculated respective broth), control 2 (PTE-

supplemented respective broth; 3 PTEs × 5 concentrations), and experimental treatments (inoculated PTE-supplemented respective broth; 3 PTEs × 5 concentrations) for each microbial strain. The composition of the reaction mixtures for each experimental tube is presented in Table 5.

Table 5
Composition of reaction mixtures (n = 3)

Treatment	RB, mL	Suspension, mL	Me solution, mL	dH_2O, mL	Total volume, mL
Blank	3.8	–	–	0.6	4.4
Control 1	3.4	0.4	–	0.6	4.4
Control 2	3.8	–	0.6	–	4.4
Experiment	3.4	0.4	0.6	–	4.4

Notes: RB – respective broth; Me – metal (Ni, Cu, or Zn) solution at five different concentrations.

Immediately after preparation, each tube was vortexed briefly to ensure homogeneous mixing, and 1 mL aliquots were collected into labelled microcentrifuge tubes for OD_{600} measurement (time point 0 h). The tubes were then incubated in a shaking incubator at 28 °C and 140 rpm for 24 h. Following incubation, 1 mL samples were collected again from each tube, and OD_{600} was measured to assess bacterial growth under different PTE concentrations.

Biocompatibility Assay

Biocompatibility/antagonism was assessed by the cross-streak method, in which the test isolate was streaked centrally on agar, incubated, and then indicator strains were streaked perpendicular to it; inhibition was evaluated from the growth-free zone at the intersection of the streaks (Balouiri et al., 2016; Velho-Pereira & Kamat, 2011).

Qualitative Analysis of Enzymatic Activity

Screening of microbial enzymatic activities was performed by streaking a single colony from a 24 h fresh culture onto fresh Petri plates containing the respective media.

Amylolytic Activity

Determination of extracellular amylase (amylolytic) activity in microbial isolates was performed using starch agar plates (composition (Ntabo et al., 2018), g L^{-1}: soluble starch, 20.0; tryptone, 5.0; yeast extract, 3.0; agar, 15.0), followed by incubation at 28 °C for 48 h. Following incubation, the plates were flooded with iodine–potassium iodide (KI) solution, which forms a dark blue-black complex with intact starch; the presence of transparent zones surrounding the colonies indicated amylase production (Amoozegar et al., 2003; Hossain et al., 2020).

Cellulolytic Activity

Screening of microbial cellulase (endoglucanase) activity was conducted using carboxymethylcellulose (CMC) agar medium (composition (Shreshtha et al., 2025), g L^{-1}: $NaNO_3$, 2.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; KCl, 0.5; CMC salt, 2.0; peptone, 0.2; agar, 17.0). The inoculated plates were incubated in a thermostat at 30 °C for 48 h (Meddeb-Mouelhi et al., 2014). Following incubation, the plates were stained with Congo red (Merck Group, Darmstadt, Germany) solution (2.0 g L^{-1}) for 10 min and subsequently destained with 1 M NaCl for 15 min; the presence of halo zones surrounding the colonies indicated cellulase production (Carder, 1986).

Lipolytic Activity

Similarly, lipolytic activity was assessed by inoculating the isolates onto Tributyrin agar (TBA) medium (composition (Carrazco-Palafox et al., 2018), g L^{-1}: peptone, 5.0; yeast extract, 3.0; agar, 15.0; tributyrin 1% v/v) followed by incubation at 28 °C for 48 h. The development of a clear zone surrounding the colony was indicative of lipase or esterase activity.

Proteolytic Activity

For determination of proteolytic activity, 1% skim milk agar (SMA) was employed (composition (Masi et al., 2021; Ntabo et al., 2018; Shreshtha et al., 2025), g L^{-1}: casein enzymatic hydrolysate, 5.0; dextrose, 1.0; yeast extract, 2.5; skim milk powder,

1.0; agar, 15.0), followed by incubation at 28 °C for 48 h. Proteolytic activity was confirmed by the appearance of proteolysis zone surrounding the colonies.

Determination of Plant-Growth Promoting Traits

Siderophore Production

Screening of microbial isolates for siderophore production was performed using an universal assay applicable to both Gram-positive (G^+) and Gram-negative (G^-) microorganisms (Schwyn & Neilands, 1987). The method was based on a colour change from blue to orange, whereby the Chrome Azurol S (CAS)–Fe^{3+} complex exhibits a blue colour, changing to orange in the presence of siderophores (Eq. 8):

$$Fe^{3+} + CAS\ dye\ (blue) + \\ +Siderophore \rightarrow Fe^{3+} + dye\ (orange) \quad (8)$$

For preparation of the CAS indicator solution, 36.3 mg of CAS was dissolved in 30 mL of dH_2O. Separately, 43.75 mg of hexadecyltrimethyl ammonium bromide (HDTMA) was dissolved in 24 mL of dH_2O, followed by the addition of 6.0 mL of Fe^{3+} solution (prepared by dissolving 27.0 mg $FeCl_3 \cdot \bullet 6H_2O$ and 83.3 µL concentrated HCl in 100 mL dH_2O). The two solutions were combined to obtain a dark blue mixture with a final volume of 60 mL. The resulting CAS indicator solution was autoclaved for sterilisation.

For basal agar medium preparation, 17.05 g of 0.1 M 3-(N-morpholino)propanesulfonic acid (MOPS), 0.285 g of NaCl, 0.17 g of KH_2PO_4, 0.055 g of NH_4Cl, and 0.285 g of L-asparagine were dissolved in 471.5 mL of dH_2O in a 1 L flask. The pH of the solution was adjusted to 6.8 using 6 M NaOH, and the volume was then adjusted to 500 mL with dH_2O. Agar (8.5 g) was added, and the medium was heated with stirring until complete dissolution, followed by autoclaving for sterilisation.

A volume of 60 mL was withdrawn from the sterilised basal agar medium to obtain a final volume of 440 mL, after which the medium was cooled to 50 °C in a water bath. The CAS indicator solution (60 mL) was prepared separately and maintained at the same temperature. Subsequently, 10 mL of 50% (w/v) glucose solution was added to the 440 mL of basal agar medium under gentle stirring, followed by 50 mL of CAS indicator solution. After thorough mixing, a total of 500 mL of blue CAS agar medium was obtained. The medium was aseptically dispensed into sterile Petri dishes (~25 mL per plate), yielding ~20 plates.

A single colony from a 24 h fresh culture plate was transferred into 10 mL test tubes containing 5 mL of the corresponding broth (NA, GYM, or YM) and incubated at 28 °C and 140 rpm for 24 h. Following incubation, the strains were streak-inoculated onto CAS agar medium using a microbiological loop. The plates were incubated at 28 °C for 7 days. The CAS reaction rate was determined by monitoring the progression of the colour-change front in the blue CAS agar. Control plates containing uninoculated blue CAS agar were incubated under identical conditions, and no colour change was observed.

Extracellular Polymeric Saccharide Production

E The ability of selected isolates to produce extracellular polymeric saccharides (EPS) was evaluated using the Congo red agar (CRA) method (Christensen et al., 1982; Freeman et al., 1989; dos Santos et al., 2021). The medium was prepared with the following composition (g L^{-1}): Congo red dye (Merck Group, Darmstadt, Germany), 0.8; brain heart infusion (BHI) broth (HiMedia Laboratories, Thane, India), 37.0; sucrose, 50.0; agar, 10.0. The composition of BHI broth was as follows (g L^{-1}): peptone, 10.0; infusion solids, 12.5; BHI powder, 5.0; dextrose, 2.0; NaCl, 5.0; Na_2HPO_4, 2.5; pH 7.4 ± 0.2. Following inoculation, the plates were incubated aerobically at 37 °C for 24 h. EPS production was confirmed by a change in colony colour to black, whereas the absence of EPS production was indicated by a stable colony colour of pink and/or red.

Indole-3-Acetic Acid Synthesis

Quantitative evaluation of indole-3-acetic acid (IAA) production by microbial isolates was performed using Salkowski's reagent and a tryptophan-supplemented nutrient broth (TNB). Salkowski's reagent was prepared by mixing 1 mL of 0.5 M $FeCl_3$ with 49 mL of 35% perchloric acid ($HClO_4$) (Phetcharat & Duangpaeng, 2012). Prior to analysis, an IAA calibration curve was created. Standard solutions were prepared according to the method described by Gang et al. (2019), maintaining a 1:1 ratio between the sample and Salkowski's reagent. Appropriate volumes of the IAA stock solution (1 mg mL^{-1}) were diluted with dH_2O to a final volume of 750 µL, followed by the addition of

750 µL of Salkowski's reagent. A blank sample was prepared using dH_2O instead of IAA. Following preparation, the mixtures were incubated in the dark for 30 min, and the optical density (OD_{536}) was measured at 536 nm using an SF-2000 spectrophotometer (OKB Spectr, Russian Federation). For calibration curve construction, OD_{536} values of standard solutions within the concentration range of 1–100 µg mL^{-1} (Ali et al., 2022) were used, yielding a linear relationship described by the equation $y = 0.0316x + 0.0586$ (R^2 = 0.98). The concentration of IAA produced by microbial cultures was calculated from OD_{536} values using Eq. 9:

$$IAA\ (\mu g\ mL^{-1}) = \frac{OD_{536} - 0.0586}{0.0316} \quad (9)$$

Specifically, a single colony from a 24 h fresh culture was transferred into 10 mL of preculture broth without L-tryptophan (nutrient broth, NB), with the following composition (g L^{-1}): peptic digest of animal tissue, 10.0; meat extract, 3.0; NaCl, 15.0; pH 7.0 ± 0.2. The cultures were incubated in a shaking incubator at 28 °C and 140 rpm for 16 h. Subsequently, 100 mL bottles containing 50 mL of TNB (NB supplemented with 0.5 g L^{-1} L-tryptophan (Mamarasulov et al., 2022)) were inoculated with 100 µL of microbial suspension and incubated under dark conditions (bottles were wrapped in aluminium foil) in a shaking incubator at 28 °C and 140 rpm for at least 7 days. At 24 h intervals, 1.5 mL of culture broth was sampled and centrifuged at 5,000 rpm for 5 min. After centrifugation, 1 mL of supernatant was mixed with 1 mL of Salkowski reagent and incubated at 30 °C in the dark for 30 min. OD_{536} was then measured, with uninoculated TNB mixed with Salkowski's reagent used as a blank. Sampling and analysis at 24 h intervals were continued until a decrease in absorbance was observed, indicating cessation of IAA production.

Data Processing

Statistical data processing for all analysed parameters was performed using RStudio desktop software (version 2026.01.0 Build 392, RStudio PBC, 2026). Initially, the dataset was assessed for outliers, followed by testing for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. If the normality assumption was not met, non-parametric tests were applied: the Kruskal–Wallis test for two-factor analysis or the Wilcoxon test for three or more factors, followed by pairwise comparisons with Bonferroni adjustment. If the homogeneity assumption was violated, Welch's correction was applied, followed by Welch's analysis of variance (ANOVA). When both normality and homoscedasticity assumptions were satisfied, one- to three-way ANOVA was performed to identify statistically significant differences between treatments, followed by Tukey's honestly significant difference (HSD) test where appropriate. In all cases, pairwise comparisons resulted in statistical groupings denoted by letter annotations, and the corresponding graphs and/or tables were generated.

Results and their discussion

Soil Contamination Profile

Inorganic Contamination

Chemical analysis of soils for PTE content revealed that none of the studied soils contained Co or Cd, whereas elevated concentrations of Ni, Zn, and As were observed (Table 6). Elevated concentrations of Cr were identified in TA, K, and TE soils, while elevated concentrations of Cu were detected in K and TE soils. In the case of Pb, elevated concentrations were observed exclusively in TE soil. Overall, it was determined that TE soil exhibited elevated concentrations of five PTEs, followed by K soil, which contained elevated concentrations of four PTEs.

In addition, assessment of the soils based on geo-accumulation index (I_{geo}) (Saleem et al., 2024) indicated that TA and K soils were moderately contaminated with As, whereas TE soil was heavily contaminated with Zn and As and extremely contaminated with Pb. Almost identical patterns were observed for the ecological risk factor (E_R) (Hakanson, 1980), with TA and K soils exhibiting moderate risk associated with As concentrations, while TE soil demonstrated high and extreme risks for Zn and Pb, respectively. Evaluation of soil toxicity using the integrated pollution index (Z_{ci}) (Vyborov et al., 2004) indicated that TA and S soils could be considered safe, whereas K and TE soils posed moderate and extreme hazards, respectively. According to the potential ecological risk index (*PERI*) (Hakanson, 1980), all soils except TE presented low risk, while the latter exhibited an extreme ecological risk.

Table 6
PTE concentrations (mg kg^{-1}) in studied soils

Element	MPC	MDL	TA soil	K soil	S soil	TE soil	*p*-value
Cr	90 [a,b]	> 12	139 ± 9.17 ab	150 ± 4.89 a	< LOD	117 ± 11.0 b	< 0.01
Co	5.0	> 12	< LOD	< LOD	< LOD	< LOD	–
Ni	20	> 12	54.4 ± 1.32 b	70.1 ± 2.00 a	31.1 ± 2.00 d	44.1 ± 4.38 c	< 0.001
Cu	33	> 5	29.0 ± 0.05 c	97.6 ± 6.94 b	27.8 ± 0.10 c	169 ± 10.6 a	< 0.001
Zn	55	> 2	68.3 ± 2.20 c	87.7 ± 2.28 b	63.1 ± 1.52 c	1 077 ± 10.9 a	< 0.001
As	2.0	> 2	10.3 ± 0.75 c	13.0 ± 0.31 b	5.24 ± 0.57 d	49.7 ± 0.81 a	< 0.001
Cd	0.5	> 2	< LOD	< LOD	< LOD	< LOD	–
Pb	32 [a]	> 15	18.8 ± 1.55 b	23.8 ± 0.36 b	18.0 ± 0.30 b	1 040 ± 33.2 a	< 0.001
Z_{ci} (Vyborov et al., 2004)			13.7	19.9	5.67	122	–
PERI (Hakanson, 1980)			73.3	97.0	40.6	682	–

Notes: MPC – maximum permissible concentrations for mobile fractions of elements (MH RK, 2021); MDL – method detection limit (mg kg^{-1}); TA – Talgar; K – Kyzylkairat; S – Saimasai; TE – Tekeli; LOD – limit of detection; [a] – MPC for total concentration; [b] – MPC values for Europe (MECR, 2016).

Organic Contamination

In addition to PTE content, K and S soils were analysed for POP concentrations (Table 7). Chemical analysis revealed elevated concentrations, exceeding MPC, of DDT metabolites, dieldrin, endosulfan α, and endrin. Among the DDT metabolites, concentrations of 2.4-DDD and 4.4-DDD exceeded MPC in both soils, while 4.4-DDT concentrations exceeding MPC thresholds were detected in S soil. Both soils exhibited comparable concentrations of 2.4-DDD; however, S soil contained significantly higher concentrations of 4.4-DDD and 4.4-DDT compared with K soil. Overall, the sum concentrations of DDT metabolites exceeded MPC by 9.28- and 13.8-fold in K and S soils, respectively.

Similarly, both soils exhibited elevated concentrations of endosulfan α and endrin, exceeding MPC by 5.22–27.6-fold and 34.0–63.5-fold, respectively. K soil contained significantly higher concentrations of endosulfan α than S soil, whereas S soil exhibited significantly higher concentrations of endrin compared with K soil. In addition, elevated concentrations of dieldrin (5.6× MPC) were detected exclusively in S soil.

Assessment of cumulative POP concentrations indicated that K soil was more contaminated than S soil overall. The largest contribution to the cumulative concentration in K soil was attributed to endosulfan α, followed by DDT metabolites. In contrast, the cumulative concentration in S soil was primarily driven by DDT metabolites, followed by endosulfan α.

Microbial Isolation & Morphology

A total of 242 isolates were obtained from the four studied soils (Table 8). The highest number of isolates was recovered from K soil, whereas the lowest number was obtained from TE soil, potentially indicating that inorganic contamination exerted a more pronounced influence on soil microbial diversity than organic contamination (Thavamani et al., 2012). Furthermore, five distinct culture media were employed for isolation, with YM medium yielding the highest number of isolates (65) and PA the lowest (25). This pattern was likely attributable to the more nutrient-rich composition of YM, specifically its enrichment with glucose (10 g L^{-1}), malt extract, and yeast extract, whereas PA contains only peptone.

Of the 242 isolates, 172 were Gram-positive (G^+) and 70 were Gram-negative (G^-). According to cell morphology, 198 isolates were classified as bacilli, 26 as cocci, and 18 as filamentous forms.

Table 7
POP concentrations (μg kg^{-1}) in studied soils

POP-pesticide	MPC	K soil	S soil	*p*-value
2.4-DDD	100	484 ± 80.7	387 ± 48.0	0.149
4.4-DDD		371 ± 17.8 b	450 ± 42.1 a	< 0.05
4.4-DDE		58.2 ± 7.79 a	33.8 ± 0.86 b	< 0.01
4.4-DDT		14.5 ± 0.05 b	512 ± 47.0 a	< 0.001
∑ DDTs		928 ± 106 b	1 382 ± 52.0 a	< 0.01
α-HCH	100	< LOD	10.8 ± 0.30	–
β-HCH		< LOD	29.6 ± 0	–
γ-HCH		< LOD	7.48 ± 0	–
δ-HCH		4.00 ± 0	8.04 ± 0	–
∑ HCHs		4.00 ± 0 b	55.9 ± 0.30 a	< 0.001
Dieldrin	0.5	< LOD	2.80 ± 0.25	–
Endosulfan α	100	2 763 ± 150 a	522 ± 24.8 b	< 0.001
Endosulfan sulfate	–	12.1 ± 1.32 a	9.09 ± 0 b	< 0.05
Endrin	1	34.0 ± 0.65 b	63.5 ± 4.97 a	< 0.001
Endrin aldehyde	–	15.8 ± 1.88 b	24.1 ± 0.82 a	< 0.01
Heptachlor	50	< LOD	5.22 ± 0.04	–
Heptachlor epoxide		5.77 ± 0 a	4.99 ± 0.19 b	< 0.01
Methoxychlor	1 600	5.49 ± 0.47 b	23.5 ± 0.44 a	< 0.001
∑ Others		2 836 ± 150 a	656 ± 31.0 b	< 0.001
∑ POPs		3 768 ± 257 a	2 094 ± 83.3 b	< 0.001

Notes: MPC – maximum permissible concentrations (MH RK, 2021); K – Kyzylkairat; S – Saimasai; LOD – limit of detection.

Table 8
Number of microbial cultures isolated from different soils using selective media

Soil	Number of isolates					
	NA	TSA	PA	GYM	YM	Total
TA	14	15	4	7	23	63
K	13	15	8	18	19	73
S	17	14	9	11	14	65
TE	9	9	4	10	9	41
All	53	53	25	46	65	242

Notes: NA – nutrient agar; TSA – tryptic soy agar; PA – pseudomonas agar; GYM – glucose yeast malt agar; YM – yeast malt agar; TA – Talgar; K – Kyzylkairat; S – Saimasai; TE – Tekeli.

Zn-Solubilisation Activity

Evaluation of Zn-solubilising activity among the 242 isolated strains on ZnO-supplemented solid media was conducted by measuring colony and halo zone diameters. Of the 242 isolates tested, 27 exhibited Zn-solubilisation potential, as indicated by the formation of halo zones around colonies (Table 9). However, variability in replication was observed; for instance, isolate TE5 developed a halo zone on only one of three plates, with a solubilisation index (SI) of 1.33. Thirteen (13) isolates formed halo zones on two out of three plates, while the remaining 13 isolates exhibited halo formation consistently across all three plates.

The intensity of Zn-solubilising activity was classified according to the following scale: SI < 1.5, weak activity; 1.5 < SI < 2.5, moderate activity; and SI > 2.5, strong activity. Among isolates exhibiting

activity on all three plates, three (3) isolates (S40, S56, and TE32) demonstrated strong Zn-solubilising activity, with SI values ranging from 2.56 to 2.77. Nine (9) isolates (TA33, S12, TA5, S20, TA15, S25, S21, S24, and S36) exhibited moderate activity, with SI values between 1.64 and 2.33, while only isolate S39 showed weak activity (SI of 1.43).

Among isolates exhibiting activity on two out of three plates, only isolate S47 demonstrated strong activity, with an SI of 3.13. Eight (8) isolates (S41, TE31, TE30, S32, K23, S43, S37, and TE15) exhibited moderate activity, whereas the remaining four (4) isolates (TE29, TE7, K45, and S54) showed weak activity.

Table 9
Solubilisation index values

No.	Isolate	SI, mean ± SD	SG	No.	Isolate	SI, mean ± SD	SG
$n = 3$, $p < 0.001$				$n = 2$, $p < 0.001$			
1	S40	2.77 ± 0.16	a	14	S47	3.13 ± 0.06	a
2	S56	2.65 ± 0.26	ab	15	S41	1.99 ± 0.26	b
3	TE32	2.56 ± 0.19	abc	16	TE31	1.75 ± 0.15	bc
4	TA33	2.33 ± 0.17	abcd	17	TE30	1.62 ± 0.23	bc
5	S12	2.17 ± 0.31	bcde	18	S32	1.60 ± 0.31	bc
6	TA5	2.16 ± 0.12	cde	19	K23	1.58 ± 0.12	bc
7	S20	1.95 ± 0.14	def	20	S43	1.54 ± 0.29	bc
8	TA15	1.83 ± 0.18	efg	21	S37	1.52 ± 0.03	bc
9	S25	1.76 ± 0.16	efg	22	TE15	1.51 ± 0.20	bc
10	S21	1.70 ± 0.10	efg	23	TE29	1.40 ± 0.09	c
11	S24	1.68 ± 0.19	efg	24	TE7	1.37 ± 0.05	c
12	S36	1.64 ± 0.18	fg	25	K45	1.25 ± 0.03	c
13	S39	1.43 ± 0.20	g	26	S54	1.17 ± 0	c

Notes: SI – solubilisation index; SD – standard deviation; SG – statistical groups.

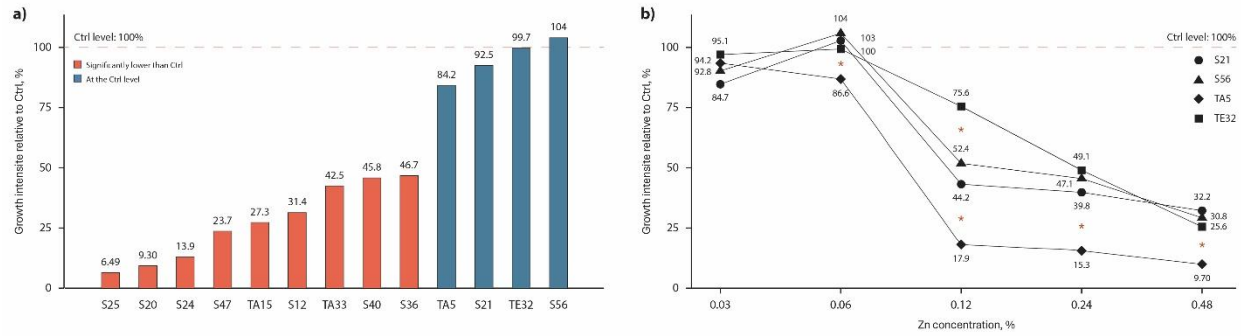
Thus, thirteen (13) isolates were selected for further investigation, specifically twelve isolates (S40, S56, TE32, TA33, S12, TA5, S20, TA15, S25, S21, S24, and S36) exhibiting moderate to strong Zn-solubilising activity consistently across all three plates, and one isolate (S47) demonstrating strong activity on two plates.

Microbial Tolerance to PTE

Pre-screening of thirteen (13) isolates, selected based on their Zn-solubilising activity, for tolerance to a Zn concentration of 0.06% on the respective media identified three isolates (S21, S56, and TE32) that exhibited growth intensity comparable to Ctrl (Fig. 2a). One isolate (TA5) showed only slight growth inhibition (15.8%), whereas the remaining nine isolates exhibited substantial growth inhibition, with reductions of up to 93.5%. Consequently, following sequential screening based on growth performance at 0.06% Zn, four isolates (S56, S21, TA5, and TE32) were selected for comprehensive evaluation of their tolerance to Ni, Cu, and Zn.

Analysis of microbial tolerance across the range of PTE concentrations (0.03–0.48%) demonstrated that, from a Zn concentration of 0.06% onwards, the growth intensities of isolates S21, S56, and TE32 were significantly higher than that of TA5 (Fig. 2b). This pattern remained consistent across all subsequent Zn concentrations. Overall, TE32 exhibited the highest growth intensity throughout the 0.06–0.24% Zn concentration range, whereas the growth intensities of S21 and S56 were nearly identical across all concentrations tested. In contrast, TA5 demonstrated tolerance to Zn only at concentrations of 0.03% and 0.06%, with growth intensity declining to 17.9% at 0.12% Zn and decreasing further to 9.70% at the higher concentrations.

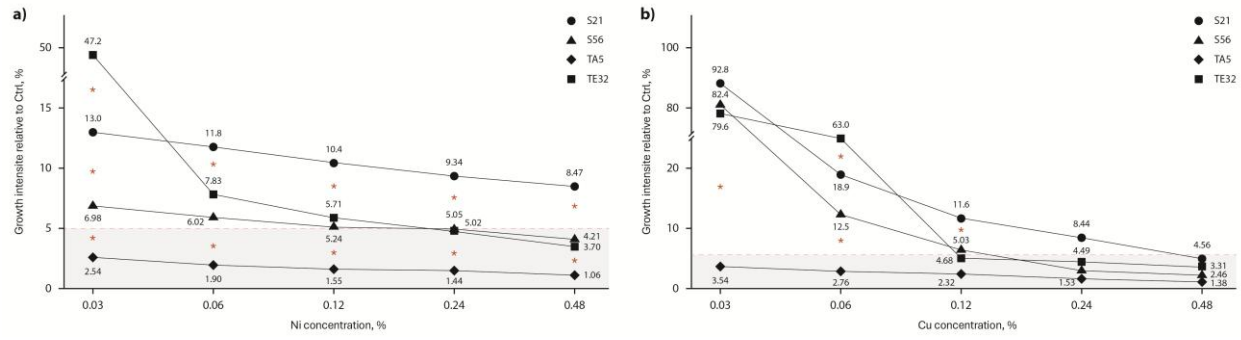
Figure 2
Growth intensity relative to the Ctrl in broth supplemented with 0.06% Zn (a) and 0.03–0.48% Zn (b)



Analysis of microbial growth intensities in Ni- and Cu-supplemented media showed that relative growth values ≤5% of the Ctrl were operationally classified as negligible residual growth, corresponding to ≥95% growth inhibition. Isolate TA5 did not exhibit tolerance to either PTE at any of the concentrations tested and also displayed the weakest tolerance to Zn. As observed for Zn tolerance, isolates S21 and S56 exhibited similar response patterns to Ni and Cu exposure; however, S21 demonstrated consistently greater and more stable tolerance to both PTEs. Specifically, under Ni-induced stress, the growth intensity of S21 declined gradually from 13.0% to 8.47% across the 0.03–0.48% Ni concentration range, indicating sustained resistance (Fig. 3a). Under Cu-induced stress, although growth intensity decreased sharply from 92.8% to 4.56%, S21 tolerated the broadest range of Cu concentrations (0.03–0.24%; Fig. 3b). Similarly, the growth intensity of S56 in Cu-supplemented medium declined markedly from 82.4% to 2.46%; however, the range of tolerated Cu concentrations was narrower (0.03–0.12%).

Isolate TE32 exhibited distinct response patterns to Ni and Cu. At the lowest Ni concentration (0.03%), TE32 displayed the highest relative growth intensity (47.2%), followed by a sharp decline to 7.83% at higher concentration (Fig. 3a). In contrast, under Cu-induced stress, TE32 demonstrated good tolerance at 0.03% and 0.06%, maintaining relative growth intensities of 79.6% and 63.0%, respectively (Fig. 3b). However, no meaningful growth was observed at the subsequent Cu concentrations. Based on the PTE tolerance profiles obtained, three isolates (S21, S56, and TE32) were selected for further investigation.

Figure 3
Growth intensity relative to the Ctrl in broth supplemented with Ni (a) and Cu (b).

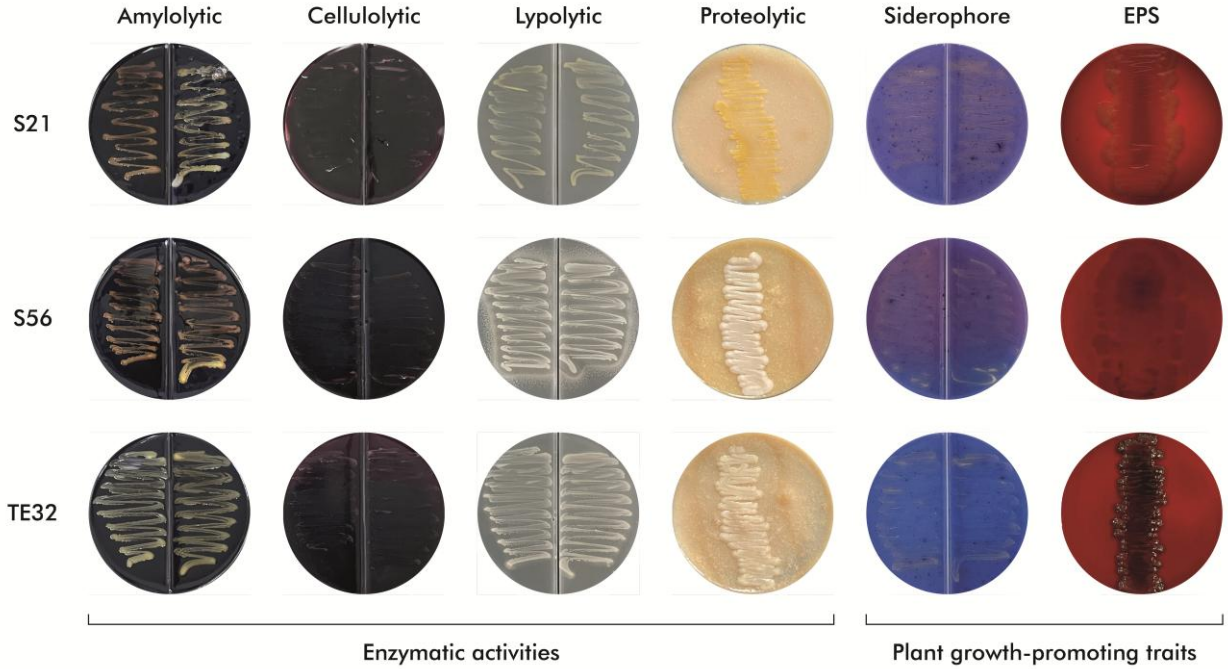


Note: The grey shaded area indicates negligible residual growth, defined as ≤5% of Ctrl. Values within this range were interpreted as near-complete growth inhibition.

Enzymatic, Plant Growth-Promoting, & Biocompatibility Profiles of Microbial Isolates

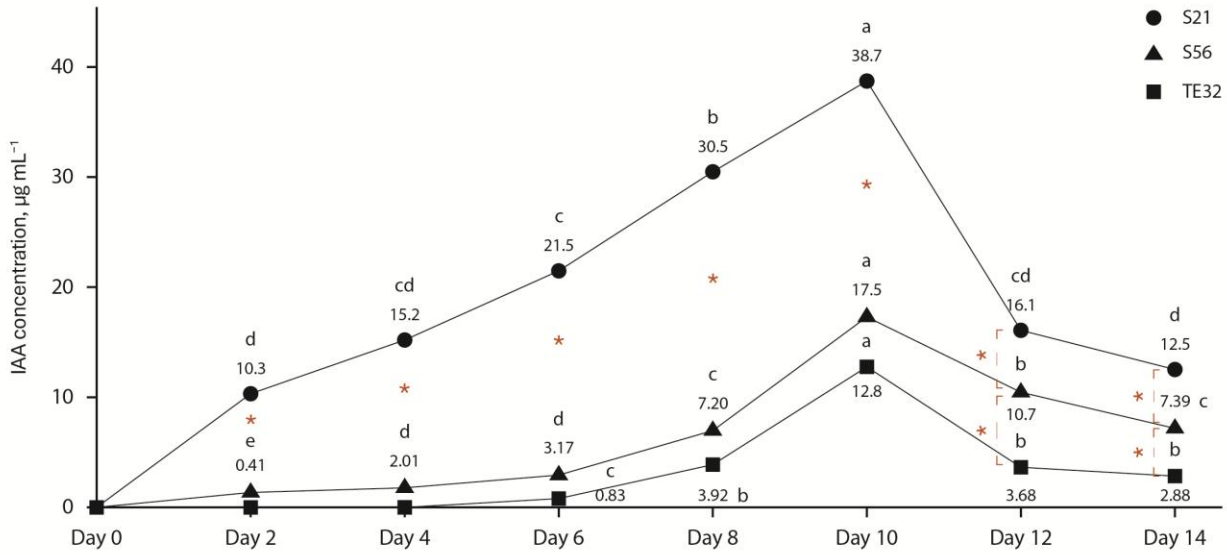
The three selected isolates were characterised qualitatively and quantitatively to establish their enzymatic and plant growth-promoting profiles. The results demonstrated that none of the isolates exhibited cellulolytic activity (Fig. 4). Amylolytic activity was detected only in isolate TE32, lipolytic activity only in S56, whereas proteolytic activity was observed in both TE32 and S56. With respect to siderophore production, a colour change from blue to reddish-purple was observed on the plates inoculated with S21 and S56, indicating positive siderophore production. Extracellular polymeric saccharide (EPS) production was detected exclusively in isolate TE32.

Figure 4
Enzymatic activities and plant-growth promoting traits of selected microbial isolates



Indole-3-acetic acid (IAA) production was detected in all three isolates; however, isolate S21 exhibited markedly superior activity (Fig. 5). IAA synthesis commenced on day 2, and the concentrations produced by S21 remained significantly higher than those of S56 and TE32 throughout the experimental period. IAA production by S56 also began on day 2 but remained low (0.41–3.17 µg mL^{-1}) until day 10. In contrast, isolate TE32 exhibited the weakest IAA-producing capacity, with production commencing only on day 6 and reaching a maximum concentration of 12.8 ± 0.88 µg mL^{-1}. For all three isolates, peak IAA concentrations were recorded on day 10, with the maximum production by S21 being 2.21- and 3.02-fold higher than that of S56 and TE32, respectively.

Figure 5
Indole-3-acetic acid production activity



To determine whether the selected isolates could be co-cultivated for the construction of a microbial consortium, biocompatibility assays were performed. The results demonstrated that all three isolates were mutually compatible.

Conclusion

The isolation of microorganisms from legacy-contaminated soils affected by potentially toxic elements (PTEs), persistent organic pollutants (POPs), or their combination resulted in a collection of 242 microbial strains. The sequential screening strategy, designed to identify isolates with high Zn-solubilising activity and strong tolerance to Ni, Cu, and Zn, enabled the selection of three promising strains: S21, S56, and TE32. Evaluation of their enzymatic activities and plant growth-promoting traits demonstrated complementary functional characteristics, suggesting that, depending on the intended application, the strains may be utilised either as a consortium for immobilisation on biochar surfaces to produce microbe–biochar complexes for subsequent application to contaminated soils to promote plant growth, or individually for the reconditioning of waste-derived biochar through reduction of its metal load. Accordingly, future research will focus on the development of microbe–biochar complexes and their validation under vegetation experiment conditions.

Conflict of interest

Authors confirm that manuscript content was approved prior to publication and declare no conflict of interests.

Funding

The study was performed within the framework of the project AP23490702 «Development of Biochar Decontamination Technology for Sustainable Waste Biomass Conversion into Value-Added Products» (abbreviated as ReChar-M2C) for 2024-2026 supported by Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Data Availability

Data are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization, A.M.; methodology, N.A., A.A., A.N., and R.B.; software, A.M.; validation, N.A., A.A., A.N., and R.B.; formal analysis, A.M.; investigation, N.A., A.A., A.N., and R.B.; resources, A.M.; data curation, N.A., A.A., and A.B.; writing–original draft preparation, A.M., N.A., A.A., and A.B.; writing–review and editing, A.M. and V.P.; visualization, A.M.; supervision, A.M. and A.B.; project administration, A.M.; funding acquisition, A.M.

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Received May 27, 2026
Accepted September 23, 2026