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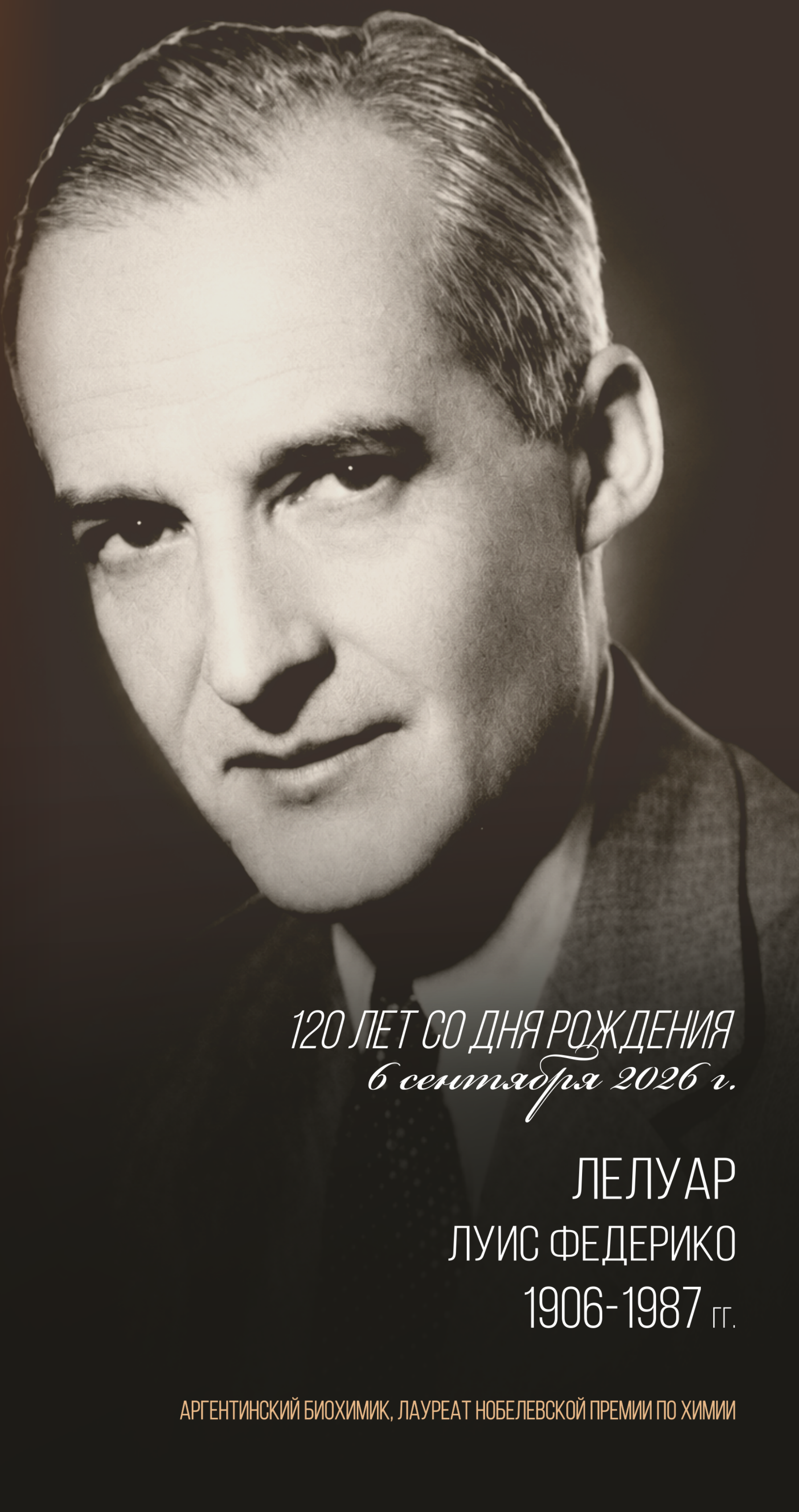
НАУЧНЫЙ ЖУРНАЛ

...ко Лелуа́р (исп. Luis Federico ...ября 1906, Париж — 2 ...87, Буэнос-Айрес) — ...кий врач и биохимик, первый ...говорящий лауреат Нобелевской премии по химии.
Окончил университет Буэнос-Айреса (1932). В 1934—1935, 1937—1943 годах работал в Институте физиологии при этом университете (с 1941 года профессор физиологии). В 1936 году в биохимической лаборатории Кембриджского университета. В 1943 году переехал в США. Работал в Вашингтонском (1944) и Колумбийском университетах (1944—1945). В 1946 году вернулся в Аргентину. С 1946 года заведующий биохимической лабораторией Института физиологии.

Член Национальной академии наук США (1960)[2], Американской академии искусств и наук (1961), Лондонского королевского общества (1972)[3], АН Аргентины. Лелуар был известен своей скромностью, сосредоточенностью и последовательностью, многие описывали его как «истинного монаха от науки»[4]. Труды по метаболизму углеводов в живых клетках, а также углеводных производных нуклеотидов. Лелуар и его коллеги изучали гликопротеины, показали основные пути метаболизма галактозы, а также пути развития галактоземии — причины появления невосприимчивости к лактозе. Совместно с группой сотрудников выделил и изучил многие ферменты углеводного обмена. Второго декабря 1970 года Лелуар получил Нобелевскую премию по химии за исследования в области метаболических путей лактозы. ... размере 80 000 долларов ... потратил на иссле...



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Isolation and characterization of soil associated bacteria

Alimova Dildora Farxod qizi
PhD student
Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan
Republic of Uzbekistan, Tashkent
E-mail: dildoraalimova2000@gmail.com
Murodullayev Dilmurod Dilshodovich
PhD student
Institute of Microbiology
Academy of Sciences of the Republic of Uzbekistan
teacher Karshi state university Karshi
Republic of Uzbekistan, Karshi
E-mail: dilmurodmurodullayev@gmail.com
Khalilov Ilkhom
doctor of Doctor of Biological Sciences
Head of Laboratory Institute of Microbiology Academy of Sciences of the Republic of Uzbekistan
Republic of Uzbekistan, Tashkent
E-mail: ilkhom2002@yahoo.com
Davranov Kakhramon
doctor of Biological Sciences, Professor
Chief Research Officer Institute of Microbiology Academy of Sciences of the Republic of Uzbekistan
Republic of Uzbekistan, Tashkent
E-mail: k-davranov@mail.ru
Rustamova Nigora Aktamovna
doctor of Biological Sciences, Senior Researcher
Institute of Microbiology Academy of Sciences of the Republic of Uzbekistan
Republic of Uzbekistan, Tashkent
E-mail: n.rustamova@yahoo.com

Выделение и характеристика почвенных бактерий

Алимова Дилдора Фарход кизи
докторант (PhD)
Институт микробиологии Академии наук Республики Узбекистан
Республика Узбекистан, г. Ташкент
Муродуллаев Дилмурод Дилшодович
докторант (PhD)
Институт микробиологии Академии наук Республики Узбекистан, преподаватель,
Каршинский государственный университет
Республика Узбекистан, г. Карши
Халилов Илхом Маматкулович
д-р биол. наук, заведующий
лабораторией, Институт микробиологии Академия Наук Республики Узбекистан
Республика Узбекистан, г. Ташкент
Давранов Кахрамон
д-р биол. наук, проф., главный научный руководитель
Институт микробиологии, Академии наук Республики Узбекистан
Республика Узбекистан, Республика Узбекистан
Рустамова Нигора Актамовна
д-р биол. наук, ст. науч. сотр. Институт микробиологии, Академии наук Республики Узбекистан
Республика Узбекистан, г. Ташкент

Abstract

The present work was undertaken to recover soil bacteria endowed with antifungal properties from agroecological soil sampled near Khonobod village in the Kashkadarya Region, and to fine-tune the output of their bioactive secondary metabolites. Employing the serial-dilution technique, upwards of fifteen bacterial isolates were secured. Each was profiled with respect to colony morphology, cellular appearance, and Gram reaction, and species-level assignment was accomplished by MALDI-TOF mass spectrometry. This procedure pinpointed six candidate strains distributed among *Bacillus atrophaeus*, *Priestia endophytica*, *Paenibacillus lautus*, *Paenibacillus* sp., and *Peribacillus frigoritolerans*. The isolates were then pitted against the phytopathogenic fungi *Alternaria* sp. and *Fusarium* spp. in a dual culture confrontation assay. Of the whole set, *Paenibacillus lautus* AD 83 mounted the most vigorous antifungal response, generating inhibition zones on the order of 13 to 18 mm. For this leading strain, the influence of medium composition, temperature, and pH on secondary-metabolite formation was mapped out. Metabolite yield peaked at 312 mg L^{-1} in a medium built on oat and corn, with the most favourable regime settling at 25 °C and pH 7.5. Collectively, the findings mark *Paenibacillus lautus* AD 83 as a compelling candidate both for the biological suppression of phytopathogenic fungi and for the formulation of ecofriendly biofungicides.

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Аннотация

Настоящая работа была предпринята с целью извлечения почвенных бактерий, обладающих противогрибковыми свойствами, из агроэкологической почвы, отобранной вблизи села Хонабад Кашкадарьинской области, а также для отладки выхода их биологически активных вторичных метаболитов. С применением метода последовательных разведений было получено свыше пятнадцати бактериальных изолятов. Каждый из них был охарактеризован по морфологии колоний, клеточному строению и реакции окрашивания по Граму, а видовая принадлежность установлена методом масс-спектрометрии MALDI-TOF. Данная процедура позволила выделить шесть перспективных штаммов, распределённых между *Bacillus atrophaeus*, *Priestia endophytica*, *Paenibacillus lautus*, *Paenibacillus* sp. и *Peribacillus frigoritolerans*. Затем изоляты были противопоставлены фитопатогенным грибам *Alternaria* sp. и *Fusarium* spp. в опыте встречных (двойных) культур. Из всего набора штамм *Paenibacillus lautus* AD 83 продемонстрировал наиболее выраженный противогрибковый эффект, образуя зоны ингибирования порядка 13–18 мм. Для этого ведущего штамма было изучено влияние состава среды, температуры и pH на образование вторичных метаболитов. Максимальный выход метаболитов (312 мг/л) достигался на среде, основанной на овсе и кукурузе, при наиболее благоприятном режиме 25 °C и pH 7,5. В совокупности полученные данные характеризуют *Paenibacillus lautus* AD 83 как убедительного кандидата как для биологического подавления фитопатогенных грибов, так и для создания экологически безопасных биофунгицидов.

Keywords: soil bacteria, Paenibacillus lautus, secondary metabolites, antifungal activity, optimization, MALDI-TOF MS, biofungicide.

Ключевые слова: почвенные бактерии, Paenibacillus lautus, вторичные метаболиты, антифунгальная активность, оптимизация, MALDI-TOF MS, биофунгицид.

Introduction

Soildwelling microbes form a cornerstone of land-based ecosystems, driving the breakdown of organic residues, the biogeochemical turnover of nutrients, the nourishment of plants, and the natural containment of phytopathogens [1]. Bacteria of the genus *Bacillus* stand out in this regard, for they both stimulate plant development and elaborate an extensive repertoire of biologically active secondary metabolites [2]. Compounds of this kind lipopeptides, polyketides, and assorted antimicrobials effectively hold back the proliferation of a wide array of plant-pathogenic fungi.

Fungal pathogens such as *Fusarium* and *Alternaria* rank among the chief culprits behind heavy yield losses and quality decline in cultivated crops [3]. Although chemical fungicides remain the conventional line of defence, their sustained use has bred fungicide resistance, polluted the environment, and harmed beneficial microbial communities. For these reasons, devising biological control measures and uncovering new antagonistic bacteria have risen to the forefront of microbiology and agricultural biotechnology.

The hunt for biologically active bacteria opens with their recovery from soil. Gauging how effectively such isolates curb phytopathogenic fungi is a pivotal stage in judging their practical value. Antagonistic bacteria commonly deploy fungal cell-wall-degrading enzymes, siderophores, volatile organic compounds (VOCs), and an assortment of secondary metabolites, all of which help rein in fungal pathogens [4]. Isolating and characterizing these metabolites and appraising what they can do biologically furnishes the scientific footing for building environmentally benign biofungicides. Accordingly, the aim of this study was to draw bacterial strains from soil, to assign them by MALDI-TOF MS, to test their antifungal action on plant-pathogenic fungi, and to obtain secondary metabolites from the most potent isolate for downstream analysis.

Materials and methods

Soil Sample Collection

Soil was gathered in March 2025 from the agroecological zone surrounding Khonobod village, Kashkadarya Region, Uzbekistan (38.852181, 65.927370). Following the envelope-sampling scheme, material was taken from a 10 cm depth, with subsamples drawn from five separate points across the survey field. Roughly 500 g of soil was sealed in sterile polyethylene bags bearing labels for the site, date, and depth of collection. The bags were carried to the laboratory and kept at 4 °C pending analysis. A portion of the soil was additionally forwarded to the Soil Composition and Repository Quality Analysis Center for physicochemical characterization [4].

Isolation of Soil Bacteria

On arrival at the laboratory, the samples were freed of stones and plant fragments. A precisely weighed 1 g aliquot was placed in a sterile tube and combined with 9 mL of sterile distilled water. To dislodge microorganisms from the soil matrix, the suspension was agitated vigorously on a Vortex MX-S mixer for 5 min and then left undisturbed for 10 min so that coarser particles could settle. This preparation constituted the 10^{-1} dilution. A 1 mL portion of the overlying liquid was next passed into a fresh tube holding 9 mL of sterile distilled water, and tenfold serial dilutions were continued as far as 10^{-5} [5]. From each dilution step, 100 µL of the bacterial suspension was distributed over the surface of sterile Petri dishes charged with a range of media by the spread-plate method. The media comprised Luria Bertani (LB) agar (yeast extract, 5 g/L; NaCl, 10 g/L; peptone, 10 g/L; agar, 15 g/L), Nutrient Agar (NA) (peptone, 5 g/L; HM Peptone B, 1.5 g/L; yeast extract, g/L; NaCl, 5 g/L; agar, 15 g/L), and Tryptic Soy Agar (TSA) (tryptone, 15 g/L; soy peptone, 5 g/L; NaCl, 5 g/L; agar, 15 g/L). Seeded plates were held at 28 °C for 24 – 48 h to permit colony formation [6]. The colonies that emerged varied markedly in appearance differing in size, outline, colour, margin, elevation, and surface. Discrete colonies were picked with care and streaked repeatedly onto fresh medium to yield pure cultures. Through successive subculturing, morphologically distinguishable colonies were separated and purified [7]. Each purified colony was taken to represent a single bacterial isolate and was retained for the ensuing morphological, biochemical, and molecular work [8, 9].

Identification of Bacterial Isolates by MALDI-TOF MS

For identification, pure cultures were grown for 24 h under suitable incubation conditions and then typed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The resulting mass-spectral fingerprints were matched against the reference entries in the MALDI-TOF MS library to establish species identity [10]. Fresh colony material was applied directly onto a polished-steel MALDI target and overlaid with α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution. Spectra were captured in linear positive-ion mode across a 2–20 kDa window, compared with the reference database in the MALDI Biotyper software, and interpreted per the manufacturer's score-based guidelines.

Evaluation of Antifungal Activity

Antifungal potential was first screened by the dual-culture antagonism assay. Five fungal test organisms were employed four Fusarium spp. strains and a single Alternaria sp. strain. The fungal pathogens were maintained on Potato Dextrose Agar (PDA) (potato extract, 200 g/L; glucose, 20 g/L; agar, 15 g/L), while the bacteria were propagated on NA. For the assay, bacterial isolates were laid down on the agar by the lawn-culture technique and then co-cultured with the fungal pathogens so that their inhibitory capacity could be assessed [11, 12]. Once the fungal colonies were fully grown, plugs bearing actively expanding mycelium were cut out aseptically with a sterile cork borer and moved onto fresh PDA plates. A 5 mm mycelial disc excised from the edge of a 7 day old culture was positioned some 25 – 30 mm from the plate centre under sterile conditions, and the bacterial strain was streaked on the far side, squarely opposite the fungal plug. All plates were incubated at 28 ± 2 °C for 7 days, alongside control plates carrying the fungal pathogen alone under matching conditions. After incubation, the zone of inhibition arising between the bacterial colony and the advancing mycelium was measured in millimetres to quantify antifungal activity [13]. To place the antagonism on a numerical footing, the radial growth inhibition (RI, %) of each pathogen was computed. This index expresses the shrinkage in fungal colony growth relative to the untreated control and is a widely adopted measure of microbial antagonistic efficacy. It was obtained from the following equation:

$$RI(\%) = \frac{R1 - R2}{R1} \times 100$$

where R1 is the radius of the fungal colony on the control plate (mm), and R2 is the radius of the fungal colony measured toward the antagonistic bacterial colony on the treatment plate (mm). Every experiment was run in triplicate, and outcomes are reported as the mean ± standard deviation (SD).

Optimization of bioactive secondary metabolite production

To refine the yield of bioactive secondary metabolites from the most active strain, *Paenibacillus lautus* AD 83, the roles of medium composition, pH, and temperature were examined. The strain was propagated in five liquid media: Nutrient Broth (NB) (peptone, 5 g/L; yeast extract, 3 g/L; NaCl, 5 g/L), Luria Bertani (LB) broth, Tryptic Soy Broth (TSB), a nitrogen-free (N-free) broth (K_2HPO_4, 0.1 g/L; KH_2PO_4, 0.4 g/L; $MgSO_4$, 0.2 g/L; NaCl, 0.1 g/L; $CaCl_2$, 0.02 g/L), and an oat–corn broth (oat flour, 15 g/L; corn flour, 15 g/L; yeast extract, 3 g/L; NaCl, 5 g/L; $CaCO_3$, 1 g/L). The impact of starting pH (5.0–8.0) and incubation temperature (20–40 °C) on metabolite output was likewise evaluated. The organism was grown under each condition and metabolite production quantified in mg/L. Medium pH was set prior to sterilization with 1 N HCl or 1 N NaOH. Temperature trials involved a 3-day incubation at each target temperature. All runs were carried out in triplicate. Following incubation, cultures were centrifuged and the metabolite concentration in the cell-free supernatant determined. From these data, the optimal medium, pH, and temperature for secondary-metabolite production by *Paenibacillus lautus* AD 83 were established.

Results and Discussion

Physicochemical analysis of the soil sample

Findings from the physicochemical assay of the soil are laid out in Table 1. Examination of the material taken from the 0–10 cm horizon showed the soil to be moderately saline. In the soil solution, sulfate (SO_4^{2-}) prevailed among the anions at 4.50 meq/L, trailed by chloride (Cl^-) at 2.00 meq/L and bicarbonate (HCO_3^-) at 0.30 meq/L; the comparatively high sulfate and chloride figures point to a sulfatechloride type of salinity. On the cation side, sodium (Na^+) dominated at 4.30 meq/L, with calcium (Ca^{2+}) and magnesium (Mg^{2+}) at 1.50 and 1.00 meq/L, respectively. This sodium predominance signals an accumulation of readily soluble salts and an advancing salinization process, which may impair soil structure by breaking down aggregates and lowering water infiltration and permeability.

The dry residue of the soil solution stood at 0.75 %, and the total soluble-salt content at 0.68 %, together confirming placement in the moderately saline class. A pH of 8.2 lent the soil a slightly alkaline character, potentially curbing the availability of certain nutrients phosphorus and micronutrients in particular to plants, while the electrical conductivity (EC) of 5.2 mS/cm reflected a fairly high load of soluble salts. Taken together, these attributes portray a moderately saline, mildly alkaline agroecological setting conditions apt to harbour stress-tolerant microorganisms able to make biologically active metabolites, and hence a promising well from which to draw microbes of agricultural and biotechnological interest.

Table 1.

Chemical characteristics of a moderately saline soil sample (0 – 10 cm depth)

Depth (cm)	HCO_3^- (meq/100 g)	Cl^- (meq/100 g)	SO_4^{2-} (meq/100 g)	Ca^{2+} (meq/100 g)	Mg^{2+} (meq/100 g)	Na^+ (meq/100 g)	Dry Residue (%)	Total Soluble Salts (%)	pH	EC (mS/cm)
0-10	0.30	2.00	4.50	1.50	1.00	4.30	0.75	0.68	8.2	5.2

Gram staining, microscopic observation, and morphological identification of bacterial isolates

Over the course of the study, more than fifteen bacterial isolates were retrieved from the collected soil. They were first told apart by colony traits—shape, margin, colour, surface texture, and size. Although the overall bacterial count in the sampled soil was rather modest, the bulk of the isolates presented as salt-tolerant organisms, in keeping with the moderately saline nature of the site. Preliminary morphological work, combined with Gram staining and microscopy, indicated that most isolates fell within the genus *Bacillus*: they proved to be Gram-positive rods, matching the hallmark features of *Bacillus* species. On the strength of the initial screening and morphological typing, six representative strains with distinctive colony characteristics were singled out for further identification and biological evaluation (Figure 1).

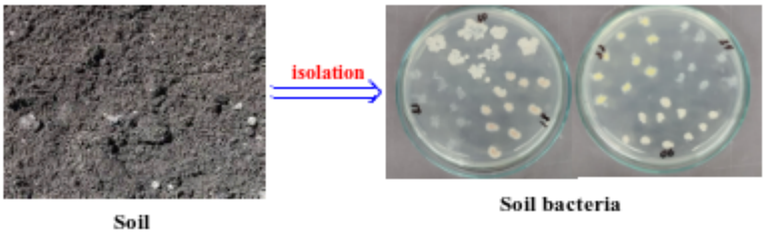

Figure 1. Isolation soil bacteria

Identification of Bacterial Isolates by MALDI-TOF MS

Table 2.

Identification by MALDI-TOF MS

Isolate code	Identified species	Identification score
AD80	*Bacillus atrophaeus*	2.31
AD81	*Priestia endophytica*	2.27
AD83	*Paenibacillus lautus*	2.34
AD87	*Paenibacillus* sp.	2.18
AD89	*Peribacillus frigoritolerans*	2.26
AD90	*Bacillus atrophaeus*	2.29

As set out in Table 2, the MALDI-TOF MS analysis placed all six isolates chiefly within the family *Bacillaceae*. In detail, isolate AD80 was identified as *Bacillus atrophaeus*, AD81 as *Priestia endophytica*, AD83 as *Paenibacillus lautus*, AD87 as *Paenibacillus* sp., AD89 as *Peribacillus frigoritolerans*, and AD90 as *Bacillus atrophaeus*. The identification scores spanned 2.18 to 2.34, denoting dependable assignment of the isolates at the genus level. The organisms so identified are familiar constituents of the soil microbiota, many of them recognized for their capacity to spur plant growth and to yield biologically active metabolites with antifungal and antibacterial effects.

Evaluation of the antifungal activity of bacteria

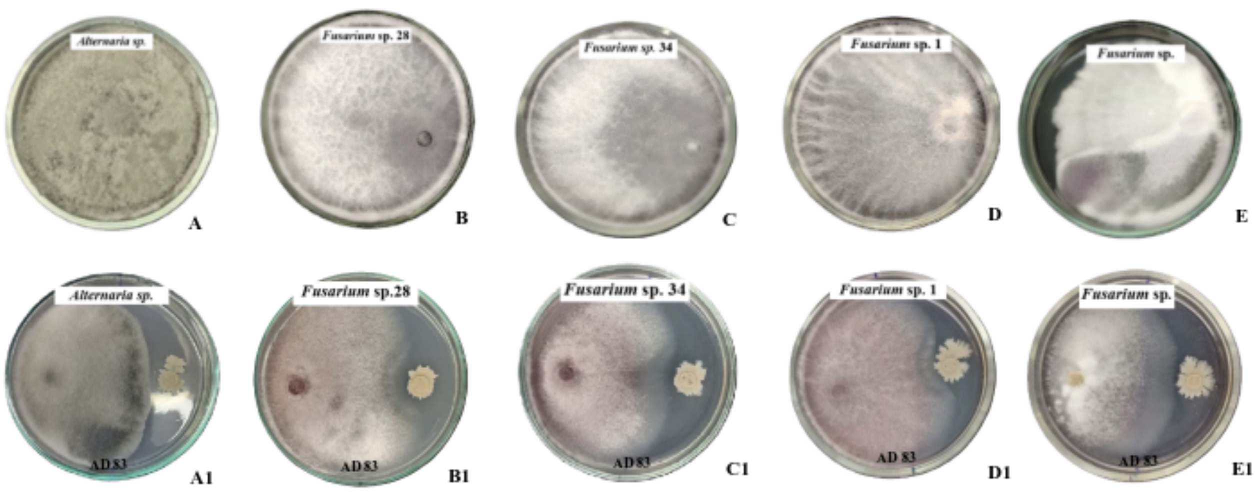

Figure 2. Antagonistic activity of Paenibacillus lautus AD 83 against phytopathogenic fungi

A-E - Control plates (fungi cultured alone): A - *Alternaria* sp.; B - *Fusarium* sp. 28; C - *Fusarium* sp. 34; D - *Fusarium* sp. 1; E - *Fusarium* sp.

A1-E1 - Dual-culture plates with *Paenibacillus lautus* AD 83: A1 - *Alternaria* sp. + *P. lautus* AD 83; B1 - *Fusarium* sp. 28 + *P. lautus* AD 83; C1 - *Fusarium* sp. 34 + *P. lautus* AD 83; D1 - *Fusarium* sp. 1 + *P. lautus* AD 83; E1 - *Fusarium* sp. + *P. lautus* AD 83.

Table 3.

Values

№	Phytopathogenic fungi	Strain name	Radial inhibition (RI %)
1	*Alternaria* sp.	*Paenibacillus lautus* AD 83	45 %
2	*Fusarium* sp. 28	*Paenibacillus lautus* AD 83	34 %
3	*Fusarium* sp. 34	*Paenibacillus lautus* AD 83	40 %
4	*Fusarium* sp. 1	*Paenibacillus lautus* AD 83	38 %
5	*Fusarium* sp.	*Paenibacillus lautus* AD 83	36 %

As the table shows, the top-performing strain, *Paenibacillus lautus* AD 83, held back every phytopathogenic fungus put to the test. The strongest suppression (45 %) fell on *Alternaria* sp., whereas the weakest (34 %) was registered against *Fusarium* sp. 28; inhibition of the remaining *Fusarium* isolates lay between 34 % and 40 %. These outcomes attest that *P. lautus* AD 83 wields pronounced antagonistic activity toward phytopathogenic fungi.

Effect of culture medium composition, ph, and temperature on secondary metabolite production

How pH, temperature, and the choice of carbon and nitrogen sources bear on the output of biologically active secondary metabolites by soil bacteria has been the subject of extensive study [14]. Bamola et al. [15] for instance, worked out the growth conditions best suited to members of the genus *Bacillus* for maximizing growth as well as antidermatophytic and antifungal activity; they found the optimum temperature for metabolite formation to be 37 °C and the optimal pH to fall between 7.0 and 8.5, under which an inhibition zone as large as 28 mm was recorded. Comparable observations were reported by Rustamova et al. [16]. In the present investigation, the best culturing conditions for *Paenibacillus lautus* AD 83 were pinned down. The strain turned out 296 mg/L of secondary metabolites at 25 °C. Among the media assessed, oat corn broth and TSB backed the highest yields, delivering 312 mg/L and 295 mg/L, respectively. The optimal pH for metabolite formation proved to be 7.5, at which the strain produced 303 mg/L.

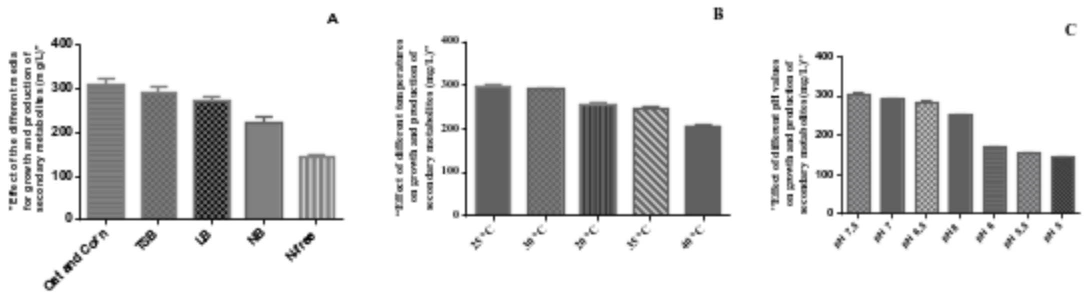

Figure 3. Effects of different culture media (A), temperature (B), and pH (C) on the growth and secondary metabolite production of the most active bacterium, Paenibacillus lautus AD 83

Conclusion

In summary, more than fifteen bacterial isolates were recovered from moderately saline, slightly alkaline agroecological soil near Khonobod village, Kashkadarya Region. MALDI-TOF MS placed six representative strains within the family *Bacillaceae*, spanning *Bacillus atrophaeus*, *Priestia endophytica*, *Paenibacillus lautus*, *Paenibacillus* sp., and *Peribacillus frigoritolerans*, with identification scores between 2.18 and 2.34. Screening by the dual-culture assay singled out *Paenibacillus lautus* AD 83 as the most effective antagonist, curbing the radial growth of the tested *Alternaria* and *Fusarium* pathogens by 34–45 %. For this strain, the conditions most conducive to secondary-metabolite production were an oat–corn medium, 25 °C, and pH 7.5, yielding up to 312 mg L^{-1}. Collectively, these results identify *Paenibacillus lautus* AD 83 as a promising microorganism for the biological control of phytopathogenic fungi and for the development of environmentally friendly biofungicides.

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Nitrate monitoring of vegetable and cucurbit products in Pop and Chust districts, Namangan region, Uzbekistan

Turayeva Fotima Rajaboyevna
Doctoral Researcher (PhD Student)
Namangan State University
Republic of Uzbekistan, Namangan
E-mail: z_turaeva78@mail.ru

Мониторинг содержания нитратов в овощной и бахчевой продукции Папского и Чустского районов Наманганской области, Узбекистан

Тураева Фотима Раджабоевна
докторант (PhD)
Наманганский государственный университет
Республика Узбекистан, г. Наманган

Abstract

This study evaluated the coverage, commodity composition, temporal dynamics, and organization of nitrate monitoring in vegetable and cucurbit products in Pop and Chust districts, Namangan Region, during 2022–2026. A retrospective dataset of 1,890 records covering eight principal commodities was analyzed. Field screening used a Greentest Eco 3F portable analyzer, with readings recorded as mg NO_3 kg^{-1}. Thirty-one records contained unambiguous numerical results. For categories with n≥3, the mean, standard deviation, and Student-t 95 % confidence interval were calculated; n=2 categories were reported as observed ranges and n=1 categories as single values. Onion, carrot, and potato represented 78.8 % of all records. The highest screening readings occurred in selected dill, coriander, cabbage, and beetroot samples. Comparison with evidence from Uzbekistan and neighboring regions supports prioritizing leafy herbs and root crops, although differences in analytical methods, seasons, and sampling designs preclude direct

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quantitative equivalence. Because paired accredited-laboratory results were unavailable, portable readings cannot establish regulatory non-compliance. A reproducible prospective programme is proposed: weekly monitoring of leafy herbs during peak season, biweekly monitoring of cucurbits during harvest, monthly monitoring of root and other vegetables, at least 10 samples per district and commodity group per round, three replicate readings per sample, and accredited laboratory confirmation whenever the screening mean reaches 80 % of the applicable limit or exceeds it.

Аннотация

Цель исследования – оценить охват, товарную структуру, временную динамику и организацию мониторинга нитратов в овощной и бахчевой продукции Папского и Чустского районов Наманганской области в 2022–2026 гг. Ретроспективно проанализировано 1890 записей по восьми основным видам продукции. Полевой скрининг выполняли портативным анализатором Greentest Eco 3F с регистрацией результата в мг NO_3/кг. Из первичного массива 31 запись содержала однозначно интерпретируемые числовые показания. Для категорий с n≥3 рассчитывали среднее, стандартное отклонение и 95 %-ный доверительный интервал Стьюдента; при n=2 приводили диапазон, при n=1 – единичное значение. Лук, морковь и картофель составили 78,8 % всех записей. Наиболее высокие скрининговые показания отмечены в отдельных пробах укропа, кориандра, капусты и столовой свёклы. Сопоставление с опубликованными для Узбекистана и соседних регионов данными подтверждает приоритет зеленных и корнеплодных культур, однако прямое количественное сравнение ограничено различиями методов, сезонов и схем отбора. Поскольку отсутствовали параллельные измерения аккредитованным лабораторным методом, результаты нельзя считать доказательством нормативного несоответствия. Предложена воспроизводимая схема дальнейшего мониторинга: еженедельный контроль зелени в сезон, контроль бахчевых каждые две недели, ежемесячный контроль корнеплодов и других овощей, не менее 10 проб на район и товарную группу за один раунд, три повторных измерения каждой пробы и обязательное лабораторное подтверждение значений, достигающих 80 % применимого предела или превышающих его.

Keywords: nitrate, vegetables, cucurbits, food safety, field screening, sanitary monitoring, Namangan Region.

Ключевые слова: нитраты, овощи, бахчевые культуры, безопасность пищевых продуктов, экспресс-контроль, санитарный мониторинг, Наманганская область.

Introduction

Food safety and sanitary control in Uzbekistan are governed by the Law of the Republic of Uzbekistan “On the Sanitary and Epidemiological Well-Being of the Population” and by national sanitary rules and hygienic regulations for food safety [1; 6].

Vegetables and cucurbit products are important sources of vitamins, minerals, and dietary fibre. However, excessive nitrogen fertilization or fertilizer application that does not follow recommended agricultural practices may promote nitrate accumulation in certain crops [3; 7; 9]. Nitrate concentrations vary with plant species, growth stage, light intensity, soil moisture and properties, fertilizer application rate, and harvest time.

International evidence shows that food safety requires continuous monitoring of chemical hazards and scientifically sound fertilizer management throughout production [2; 10]. Recent research also emphasizes that dietary nitrate should be interpreted in relation to its food source, exposure level, and broader dietary context [8]. Routine monitoring of vegetable and cucurbit products sold at retail outlets, production sites, and public institutions is therefore an important component of regional food-safety management.

This study aimed to assess the coverage, commodity composition, annual dynamics, available spatial metadata, and limitations of spatial analysis of nitrate monitoring in Pop and Chust districts from 2022 to 2026 and to summarize clearly documented rapid-screening results using descriptive statistics.

Materials and Methods

The investigation was a retrospective analysis of nitrate-monitoring records generated from January 2022 through the available part of 2026. The sampling frame comprised Pop City and Pop District, Chust City and Chust District, the Gurumsaroy area, farmers' markets, retail outlets, preschool educational institutions, selected public institutions, and agricultural production sites in Namangan Region, Uzbekistan. Records covered potato, onion, carrot, cucumber, tomato, cabbage, melon, watermelon, beetroot, turnip, pumpkin, pepper, leafy vegetables, apple, and other products. The unit of analysis was one archived monitoring record. Eight commodities with sufficiently complete annual counts were retained for coverage analysis; all 31 records with an unambiguous numerical nitrate result and unit were retained for concentration summaries. Records lacking a readable value, product identity, or interpretable measurement unit were excluded from the concentration analysis but not silently recoded.

Field investigations were conducted on 24 June and 1, 2, and 15 July 2026 in the Sang village area of Pop District, Namangan Region, Uzbekistan, at 40°50′31.61″ N, 71°10′42.13″ E and 332 m above sea level. This location was included among the agricultural production sites represented in the monitoring archive. The coordinates and elevation identify the field-survey locality; they should not be interpreted as coordinates for every market, institution, or production record in the retrospective dataset. Commodity names and spelling variants were harmonized before analysis. Records were grouped by calendar year and commodity; absolute counts and percentage shares were calculated for the eight principal commodities. The 2026 dataset covered an incomplete calendar year and is marked "2026*". Available site descriptions were standardized into district/city, market or retail, preschool/public institution, and production-site categories. Because the historical archive did not preserve a complete district-by-site-type cross-tabulation or coordinates for every record, unsupported spatial estimates and retrospective mapping were not performed.

Field screening was performed with a Greentest Eco 3F portable nitrate analyzer (Greentest, Hong Kong). For the historical measurements, an intact representative product was selected, visible surface contamination was removed, and the probe was inserted directly into edible tissue; the stabilized display value was transcribed in mg NO_3 kg^{-1}. The commodity profile was selected in the device software before measurement, and its embedded threshold served only as a screening trigger. The archive did not consistently document probe depth and position, sample temperature, cleaning between samples, calibration checks, replicate readings, certified reference materials, or matrix-specific recovery. These omissions are reported because they prevent exact analytical replication and retrospective estimation of repeatability, uncertainty, and bias. A prospective replication protocol should standardize edible-tissue position and probe depth according to the manufacturer's instructions, clean and dry the probe between samples, equilibrate samples to a common temperature, record the selected device profile and calibration status, obtain three readings at separate positions, and analyze a retained homogenized portion by a validated ion-selective, spectrophotometric, or ion-chromatographic method in an accredited laboratory.

Validation Status and Prospective Validation Protocol

No paired portable-analyzer and accredited-laboratory dataset was generated during the retrospective monitoring period. Consequently, correlation, systematic bias, repeatability, recovery, and matrix-specific measurement error cannot be calculated from the available archive without creating unsupported data. The Greentest Eco 3F values are therefore classified strictly as preliminary screening readings. A prospective validation study should include 10 – 15 samples spanning low, intermediate, and high nitrate concentrations and representing leafy herbs, root vegetables, fruit vegetables, and cucurbits. Each sample should receive three standardized portable readings and parallel analysis of a retained homogenized portion by a validated ion-selective, spectrophotometric, or ion-chromatographic method in an accredited laboratory. The analysis should report the correlation coefficient, mean paired bias with a 95 % interval, repeatability as coefficient of variation, and matrix-stratified error. Until that validation is completed, the device results must not be used to determine legal compliance or analyzer accuracy. Statistical analysis was descriptive and commodity-specific. For $n \geq 3$, the arithmetic mean, sample standard deviation (denominator $n-1$), and two-sided 95 % confidence interval were calculated as mean ± $t_{0.975,n-1} \times SD/\sqrt{n}$. For $n=2$, only minimum-maximum range was reported; for $n=1$, the observed value was reported without dispersion statistics. Formal normality testing was not used because category sizes of 1 – 6 provide little diagnostic power. Calculations were checked against the tabulated source values. No missing value was imputed, no outlier was deleted solely because of magnitude, and no prevalence of regulatory non-compliance was estimated. Screening values were interpreted against published commodity benchmarks only for prioritization; official decisions require a current legal limit and confirmatory laboratory result.

Figure 1. On-site nitrate screening of melon and watermelon samples using a Greentest Eco 3F portable nitrate analyzer

Source: Author's photographs taken during field monitoring.

Results and Discussion

During data verification, records initially grouped for 2022 and 2023 were reorganized into separate annual datasets. Temporal patterns were therefore evaluated for 2022, 2023, 2024, 2025, and the available portion of 2026.

The monitoring-coverage results and descriptive statistics for the rapid-screening observations are presented below.

Table 1.

Monitoring coverage of eight major vegetable and cucurbit commodities, 2022–2026

Commodity	2022	2023	2024	2025	2026*	Total	Share (%)
Potato	112	95	119	114	45	485	25.7
Onion	110	110	129	122	47	518	27.4
Carrot	95	113	119	116	43	486	25.7
Cucumber	5	16	24	22	6	73	3.9
Tomato	13	22	28	26	8	97	5.1
Melon	10	5	9	13	11	48	2.5
Watermelon	12	6	10	14	14	56	3.0
Cabbage	7	15	48	41	16	127	6.7
Total	364	382	486	468	190	1890	100.0

Source: Compiled by the author from primary nitrate monitoring records. Note: 2026 represents an incomplete calendar year.*

A total of 1,890 monitoring records were compiled for the eight selected commodities. Onion accounted for the largest share (518 records; 27.4 %), followed by carrot (486; 25.7 %) and potato (485; 25.7 %). Together, these three commodities represented 78.8 % of all records, probably reflecting their widespread year-round consumption and continuous availability in commercial markets.

Annual monitoring coverage was highest in 2024 (486 records), followed by 2025 (468), 2023 (382), and 2022 (364). The 190 records available for 2026 cover only part of the calendar year; therefore, the lower total for 2026 should not be interpreted as a decline in monitoring activity

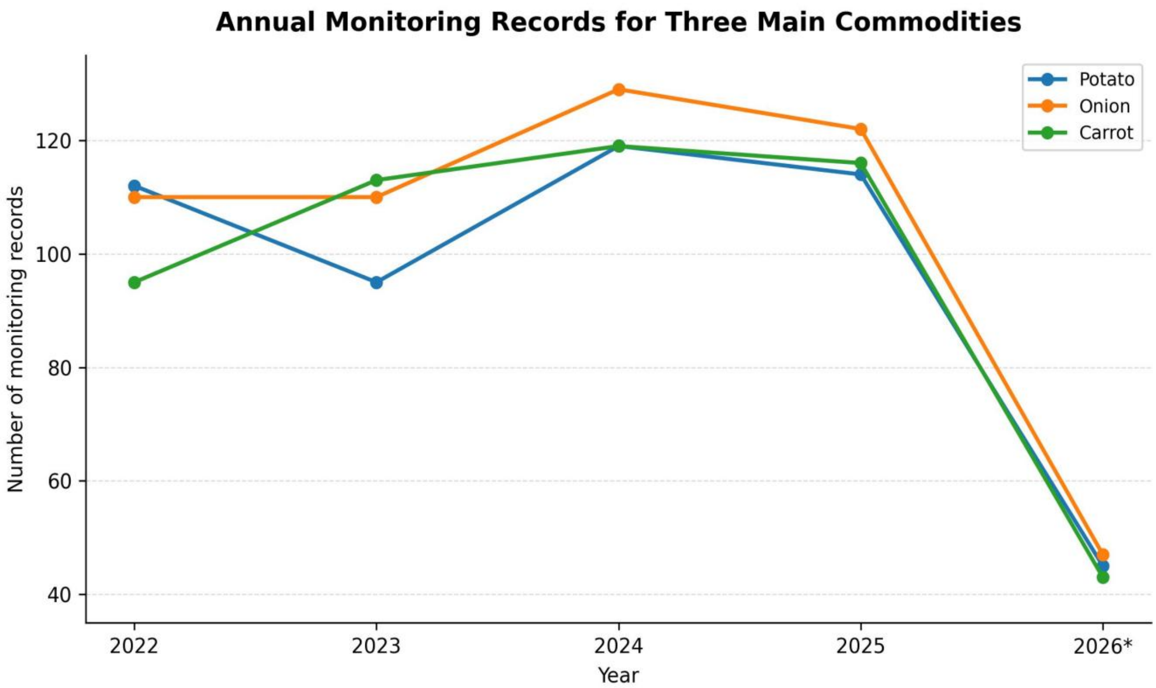

Figure 2. Annual number of nitrate-monitoring records (records per year) for potato, onion, and carrot, 2022–2026. Each colour identifies one commodity; 2026* is an incomplete calendar year

Source: Prepared by the author based on primary monitoring records

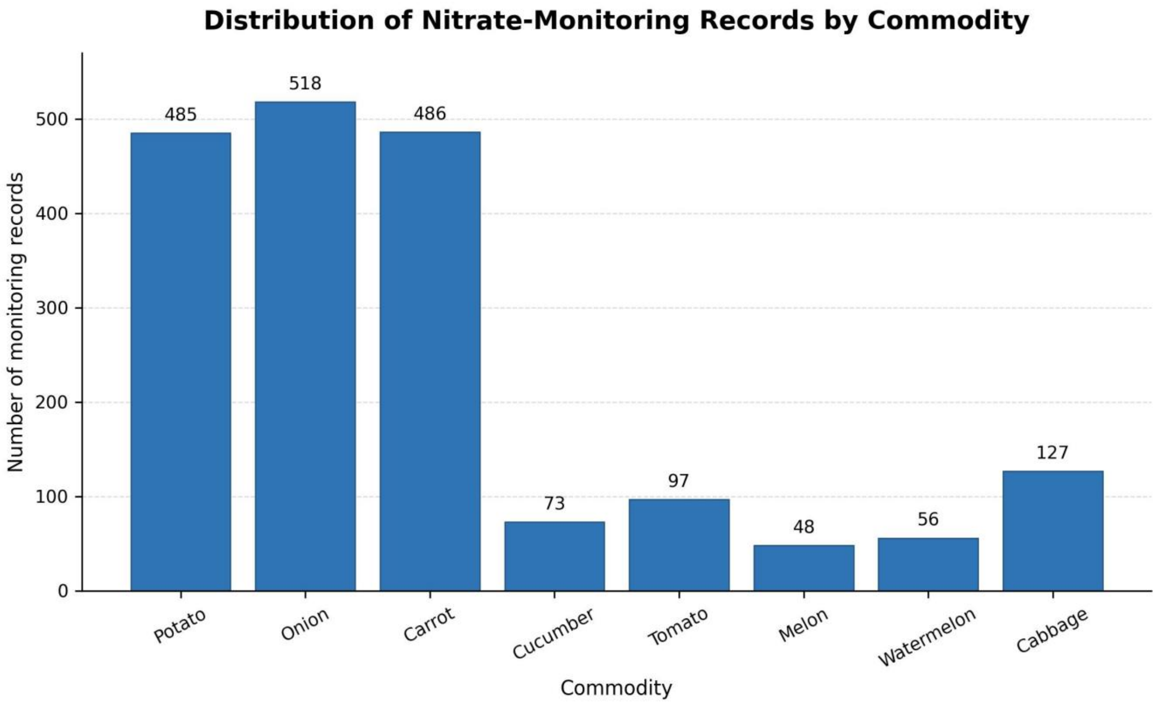

Figure 3. Number of nitrate-monitoring records by vegetable and cucurbit commodity (total n = 1,890)

Source: Prepared by the author based on primary monitoring records

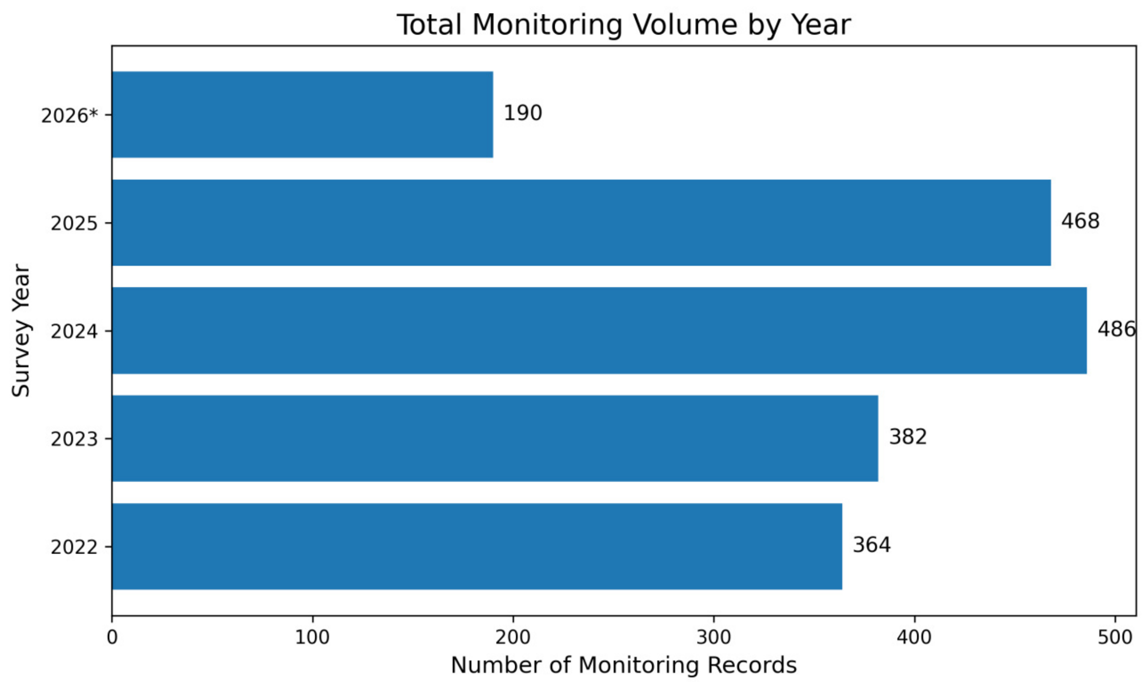

Figure 4. Annual total number of nitrate-monitoring records (records per year) for eight selected commodities, 2022–2026. 2026* is an incomplete calendar year

Source: Prepared by the author based on primary monitoring records.

Annual patterns for the principal commodities were broadly similar. In 2024, onion had the largest number of records (129), while potato and carrot each had 119. Although the number of records decreased slightly in 2025, overall monitoring coverage remained high.

Among the remaining commodities, cabbage represented 6.7 % of all records, followed by tomato (5.1 %), cucumber (3.9 %), watermelon (3.0 %), and melon (2.5 %). The smaller numbers for melon and watermelon probably reflect their seasonal production and market availability.

Table 2.

Descriptive summary of rapid nitrate-screening readings and screening comparison with historical reference levels published in SanPiN 2.3.2.560-96

Commodity	n	Descriptive result (mg kg⁻¹)	95% CI for mean	Historical reference level in SanPiN 2.3.2.560-96 (mg kg⁻¹)	Screening comparison
Onion	4	Mean 67.6; SD 25.6	26.9-108.3	80	Mean below; 95% CI crosses the reference level
Dill	2	Range 2010-2105	Not estimated	2000 open field; 3000 protected cultivation	2010-2105: above open-field, below protected-cultivation level
Coriander	2	Range 2010-2154	Not estimated	2000 open field; 3000 protected cultivation*	2010-2154: above open-field, below protected-cultivation level*
Tomato	2	Range 17-174	Not estimated	150 open field; 300 protected cultivation	167-174: above open-field, below protected-cultivation level
Cabbage	2	Range 2105-2204	Not estimated	900 early; 500 late	2105-2204: above both seasonal levels
Potato	2	Range 271-707	Not estimated	250	271-707: above the reference level
Cucumber	3	Mean 93.0; SD 72.5	0–273.1†	150 open field; 400 protected cultivation	Mean below; 95% CI crosses the open-field level
Melon	6	Mean 160.7; SD 121.1	33.6-287.8	90	Mean and observed maximum above the reference level
Muskmelon	3	Mean 196.2; SD 145.3	0–557.2†	90	Mean and observed maximum above the reference level
Apple	1	Single value 3.49	Not estimated	60	Single reading below the reference level
Carrot	2	Range 113-142	Not estimated	400 early; 250 late	113-142: below both seasonal levels
Beetroot	1	Single value 2560	Not estimated	1400	Single reading above the reference level
Bell pepper	1	Single value 349	Not estimated	200 open field; 400 protected cultivation	349: above open-field, below protected-cultivation level

Note: *The numerical values in this table are historical screening comparators taken from Table 2 of SanPiN 2.3.2.560 – 96: potato 250; early/late carrot 400/250; open/protected tomato 150/300; open/protected cucumber 150/400; beetroot 1400; onion 80; leafy vegetables 2000/3000; melon 90; apple 60; early/late cabbage 900/500; and open/protected sweet pepper 200/400 mg* NO_3 *kg*$^{-1}$*. Commodity-specific numerical maximum permissible levels from Uzbekistan SanPiN No. 0079 – 24 were not available in the retrospective source materials and could not be verified from the study archive; therefore, no value in this table is presented as a current Uzbekistan legal limit.*
**Coriander was not listed separately in the photographed historical table and was provisionally grouped with leafy herbs under the ‘and others’ wording. Greentest readings were not confirmed by an accredited laboratory, so the comparisons are used solely to prioritize confirmatory sampling*

and cannot establish legal non-compliance. †Because nitrate concentrations cannot be negative, negative lower confidence bounds produced by the small-sample normal-theory calculation are displayed as zero; the intervals remain highly uncertain.

The revised analysis avoids means and standard deviations for categories with n<3. For n≥3, uncertainty was substantial: the 95 % confidence intervals were wide, especially for cucumber and muskmelon. The highest individual screening readings occurred in dill, coriander, cabbage, and beetroot. Because cultivation system, harvest timing, and confirmatory laboratory results were not consistently recorded, the comparator column supports risk prioritization only.

These findings require cautious interpretation because several commodity categories contained few observations, the records were retrospective, and confirmatory laboratory analyses were unavailable. The results therefore cannot be generalized to all vegetable and cucurbit products in the study area.

Regional and Hygienic Assessment

The programme included Pop City and Pop District, Chust City and Chust District, the Gurumsaroy farmers' market, preschool institutions, public institutions, and agricultural production sites. However, the archived aggregate dataset did not retain a complete district-by-site-type cross-tabulation for all 1,890 records. A retrospective map or numerical district comparison would therefore create unsupported precision. Future records should capture coordinates and site type so that district-specific rates, confidence intervals, and maps can be produced. The only complete georeferenced field-survey record is presented in Table 4; it documents the Sang village production-field locality and the 2026 survey dates without implying coordinates for other archived records.

Although a portable nitrate analyzer is practical for rapid field screening, its readings should not be treated as evidence of official hygienic compliance. Results should be assessed against the applicable commodity-specific limits, and readings near or above those limits should be confirmed using a standardized analytical method in an accredited laboratory.

For reliable comparison and interpretation, each measurement record should include the sample identification code, commodity and cultivar, sampling date and location, site type, analyzer model, calibration date, number of replicate measurements, mean value, measurement unit, and applicable regulatory limit.

Table 3.

Minimum data fields recommended for standardized nitrate-monitoring records

Required Data Field	Scientific and Practical Significance
Sample identification code, commodity type, and cultivar	Enables accurate sample identification and facilitates repeat verification.
Sampling date, location, and type of sampling site	Allows the assessment of seasonal and spatial variation.
Analyzer model and calibration date	Improves the evaluation of the metrological reliability of measurements.
At least three replicate measurements	Allows calculation of the mean value and measures of variability.
Measurement unit and applicable regulatory limit	Required for assessing hygienic compliance according to commodity-specific standards.

Required Data Field	Scientific and Practical Significance
Confirmatory laboratory method	Provides definitive verification of screening results and supports regulatory decisions.

Table 4.

Available georeferenced field-survey locality in the retrospective archive

Recorded field	Recorded value
District	Pop District
Locality	Sang village
Site type	Agricultural production field
Survey dates	24 June; 1, 2 and 15 July 2026
Coordinates	40°50′31.61″ N; 71°10′42.13″ E
Elevation	332 m a.s.l.

Note: This was the only complete coordinate-and-elevation record available for the added 2026 field survey. Comparable georeferenced records for Chust District and for every market or institution were not available; therefore, a retrospective district-level map or statistical spatial comparison was not produced.

Table 5.

Proposed stratification of future nitrate monitoring by district and site type

Stratum	Routine frequency	Minimum samples per monitoring round	Required spatial record
Leafy herbs at markets/ retail	Weekly in peak season	≥10 per district	District, market/outlet, coordinates
Root crops	Monthly	≥10 per district	District, producer/vendor, coordinates
Cucurbits	Every 2 weeks in harvest season	≥10 per district	District, field/market, coordinates
Other vegetables	Monthly	≥10 per district	District, site type, coordinates
Preschool/public institutions	Monthly; immediate follow-up after trigger	≥5 per institution type per district	Institution code and coordinates
Production fields	At pre-harvest and market entry	≥5 fields per major commodity per district	Field code, cultivar, cultivation system, coordinates

Note: These targets are operational recommendations for balanced surveillance, not a claim that the retrospective dataset met them.

Interpretation of Findings

Operational Response Algorithm

1) Collect the minimum stratum-specific sample; 2) obtain three portable readings from standardized edible-tissue positions; 3) if the mean is below 80 % of the applicable comparator, record as routine screening; 4) if the mean is 80–100 % of the comparator, repeat sampling from the same lot and schedule laboratory confirmation; 5) if the mean exceeds the comparator, retain a

traceable confirmatory sample and submit it promptly to an accredited laboratory; 6) base any regulatory decision only on the validated laboratory result; 7) after confirmation, intensify sampling at the same supplier/site and review fertilizer, irrigation, cultivar, and harvest records.

The findings indicate that nitrate monitoring in Pop and Chust districts has operated as a multiyear surveillance programme covering diverse sampling locations. The predominance of records for onion, carrot, and potato probably reflects their stable year-round availability and high consumption.

Nitrate accumulation depends on crop species, nitrogen source and rate, light intensity, soil moisture, cultivar, and harvest timing [3-5; 7; 9]. Leafy herbs can accumulate more nitrate because nitrate reduction is constrained under low light and when nitrogen supply exceeds immediate growth demand. High dill and coriander readings may therefore reflect fertilizer timing, protected cultivation, shading, irrigation regime, cultivar, or time of harvest; these explanations are plausible mechanisms, not causal findings, because the historical records lacked agronomic covariates. Cabbage and beetroot are also recognized accumulator crops, making them appropriate targets for intensified sampling. Risk reduction should emphasize soil-test-based nitrogen plans, split fertilizer applications, avoidance of excessive late nitrogen, adequate light and balanced irrigation, and harvest timing appropriate to the crop.

Portable nitrate analyzers offer a rapid and practical approach to field and market screening, but readings may be affected by sample temperature, moisture content, tissue characteristics, probe placement, and calibration status. Accordingly, readings near or above applicable limits should not be considered conclusive evidence of non-compliance unless confirmed by a standardized laboratory method.

Monitoring coverage and hygienic compliance are distinct outcomes. A large number of records indicates surveillance intensity, not the proportion of compliant or non-compliant products. A defensible compliance assessment requires commodity-specific regulatory limits and confirmation by an accredited laboratory.

Comparison with evidence from Uzbekistan and neighboring regions strengthens—but also qualifies—the interpretation. Normakhmatov and Abdurayimov [11] summarized nitrate risks in Uzbek fruit and vegetable production and identified dill, beetroot, radish, and turnip as crops prone to accumulation; they also emphasized nitrogen management and soil monitoring. The elevated dill and beetroot screening readings in the present dataset are directionally consistent with that national assessment. However, their article was primarily a normative and literature-based analysis rather than a field dataset, so it cannot be used as an independent numerical validation of the present measurements.

A regional laboratory study in Uzhgorod used homogenized samples and an ion-selective laboratory method, with 25 samples per vegetable and explicit seasonal sampling [12]. It reported more frequent exceedances in early-season cucumbers, carrots, tomatoes, peppers, and cabbage. By contrast, the present study found the strongest screening signals in herbs, cabbage, and beetroot, while cucumber had a lower mean with a wide confidence interval. The difference may reflect season, cultivation system, sample composition, and analytical method rather than a true geographic contrast. An Iranian study using laboratory measurements likewise found marked variation among vegetable types and between fresh and cooked products [13], supporting the need to record matrix, season, and processing state. Published Uzbek field datasets with paired portable and reference laboratory measurements remain scarce; therefore, this study contributes regional surveillance evidence but should not be treated as a validated exposure or compliance survey.

Study Limitations

This study used retrospective monitoring records. Essential metadata-including cultivation system, cultivar, fertilizer and irrigation history, harvest time, exact coordinates, site type, replicate readings, calibration checks, and paired laboratory results-were incomplete. Category-specific samples were small (n=1 – 6), formal normality testing was uninformative, and the 2026 data covered only part of the year. The absence of a complete district-by-site-type cross-tabulation prevented a defensible retrospective spatial map.

Accordingly, the portable-analyzer results are preliminary screening observations. They cannot be used to estimate prevalence, determine legal compliance, quantify analyzer accuracy, or infer causes of nitrate accumulation. These questions require prospective stratified sampling, validated laboratory confirmation, and complete agronomic and spatial metadata. The absence of archived paired samples is an irrecoverable limitation of the retrospective design; coefficients of correlation and bias can only be generated in a new prospective validation experiment and were therefore not estimated in this article.

Conclusion

Regular nitrate surveillance was documented in Pop and Chust districts during 2022–2026. Among 1,890 records, onion, carrot, and potato accounted for 78.8 %. The 31 interpretable portable readings identified leafy herbs, cabbage, beetroot, and selected cucurbit samples as priorities for confirmatory monitoring, but they did not establish regulatory non-compliance.

Statistical reporting was corrected by using 95 % confidence intervals only for n≥3, observed ranges for n=2, and single values for n=1. Monitoring should be optimized as follows: leafy herbs weekly during peak production; melons and other cucurbits every two weeks during harvest; root crops and other vegetables monthly; and preschool or public-institution supplies monthly with immediate follow-up after a trigger. Each monitoring round should include at least 10 samples per district and priority commodity group, while institution-based rounds should include at least five samples per institution type per district. Each sample should receive three standardized portable readings. A mean at 80–100 % of the applicable limit should trigger repeat lot sampling and scheduled laboratory confirmation; a mean above the limit should trigger prompt accredited-laboratory confirmation before any regulatory action. Priority commodities are dill, coriander, cabbage, beetroot, potato, melon, and muskmelon. Complete recording of cultivar, cultivation system, fertilizer and irrigation history, harvest time, coordinates, calibration status, and confirmatory results is necessary for defensible temporal and spatial analysis.

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DIGITAL METHODS

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Real-time classification and compliance assessment of inorganic compounds in agricultural groundwater using smart hydrogeological IoT networks

Tillaeva Dilobar Abdugaffar kizi
master of Chemical science Andijan state technical institute
Republic of Uzbekistan, Andijan
E-mail: feruzas093@gmail.com

Классификация и оценка соответствия неорганических соединений в сельскохозяйственных подземных водах в режиме реального времени с использованием интеллектуальных гидрогеологических сетей Интернета вещей (IoT)

Тиллаева Дилобар Абдугаффар кызы
магистр хим. наук, Андижанский государственный технический институт
Республика Узбекистан, г. Андижан

Abstract

Agricultural groundwater may contain inorganic constituents originating from natural mineral dissolution, fertilizers, and irrigation return flows. Conventional laboratory monitoring is accurate but often limited by delayed results and insufficient sampling frequency. This study proposes a smart hydrogeological Internet of Things (IoT) network for real-time monitoring, classification, and compliance assessment of groundwater used for irrigation. The system integrates multiparameter sensors, wireless communication, geospatial databases, and machine-learning algorithms to monitor key water-quality parameters, including pH, electrical conductivity, total dissolved solids, major

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ions, and selected potentially toxic elements. Collected data are automatically validated, classified using hydrochemical and irrigation-water quality indicators, and compared with established standards. The framework can generate early-warning notifications and digital compliance reports when permissible limits are exceeded. This approach reduces monitoring delays, improves data traceability, and supports timely decision-making for sustainable agricultural water management.

Аннотация

Сельскохозяйственные подземные воды могут содержать неорганические компоненты, происходящие из естественного растворения минералов, удобрений и возвратных оросительных потоков. Обычный лабораторный мониторинг точен, но часто ограничен задержкой результатов и недостаточной частотой отбора проб. В данном исследовании предлагается интеллектуальная гидрогеологическая сеть Интернета вещей (IoT) для мониторинга в реальном времени, классификации и оценки соответствия подземных вод, используемых для орошения. Система интегрирует многопараметрические датчики, беспроводную связь, геопространственные базы данных и алгоритмы машинного обучения для мониторинга ключевых показателей качества воды, включая водородный показатель, электрическую проводимость, общее содержание растворённых веществ, основные ионы и отдельные потенциально токсичные элементы. Собранные данные автоматически проверяются, классифицируются с использованием гидрохимических показателей и показателей качества оросительной воды и сравниваются с установленными стандартами. Платформа может генерировать предупреждающие уведомления и цифровые отчёты о соответствии при превышении допустимых пределов. Данный подход снижает задержки мониторинга, повышает отслеживаемость данных и поддерживает своевременное принятие решений для устойчивого управления сельскохозяйственными водными ресурсами.

Keywords: Agricultural groundwater, inorganic constituents, Internet of Things, real-time monitoring, hydrochemical classification, irrigation-water quality, machine learning, compliance assessment.

Ключевые слова: Сельскохозяйственные подземные воды, неорганические компоненты, Интернет вещей, мониторинг в реальном времени, гидрохимическая классификация, качество оросительной воды, машинное обучение, оценка соответствия.

1. Introduction

Groundwater is an important source of irrigation, particularly in arid and semi-arid regions with limited surface-water resources. Approximately 69 % of global groundwater abstraction is used by agriculture, and groundwater supplies nearly one-quarter of irrigation water worldwide [1]. Therefore, its availability and chemical quality are essential for sustainable agricultural production [2].

Groundwater chemistry is influenced by natural processes, including mineral dissolution, evaporation, ion exchange, and water–rock interactions. Agricultural activities, particularly fertilizer application and irrigation return flows, may increase nitrate, ammonium, chloride, sulfate, and potentially toxic element concentrations [3,4]. Long-term use of unsuitable groundwater can reduce crop productivity, increase soil salinity, and degrade irrigated land.

Irrigation-water suitability is commonly assessed using electrical conductivity (EC), total dissolved solids (TDS), pH, sodium adsorption ratio (SAR), residual sodium carbonate (RSC), major ions, and potentially toxic elements. These indicators characterize salinity, sodicity, infiltration, and toxicity risks. According to FAO guidelines, excessive salinity and sodium may negatively affect plant water availability and soil structure [5].

Conventional groundwater monitoring relies on periodic sampling and laboratory analysis. Although reliable, it is time-consuming and may fail to detect short-term chemical changes. Internet of Things (IoT) technologies provide continuous monitoring through sensors, wireless communication, and cloud-based data management, enabling real-time measurements and early warnings when established limits are exceeded. However, many existing systems monitor only basic parameters and do not integrate sensor data with hydrochemical classification and agricultural water-quality standards [6,7].

Therefore, this study aims to develop a smart hydrogeological IoT network for the real-time monitoring, classification, and compliance assessment of inorganic constituents in agricultural groundwater [8]. The proposed framework integrates multiparameter sensors, hydrochemical indices, geospatial analysis, and machine-learning algorithms to evaluate salinity, sodicity, ionic composition, and contamination risks. In this study, certification refers to automated preliminary conformity assessment rather than formal regulatory certification, providing early warnings and supporting evidence-based groundwater management [9,10].

2. Materials and Methods

2.1. Study Design and Monitoring Sites

A real-time groundwater monitoring framework was designed for agricultural areas where groundwater is used as the principal or supplementary source of irrigation. Monitoring wells were selected to represent different hydrogeological and agricultural conditions, including variations in aquifer depth, crop type, fertilizer application, soil salinity, and proximity to drainage systems. The geographical coordinates and elevations of all monitoring points were recorded using a Global Positioning System device.

Before installation of the Internet of Things (IoT) units, each well was purged to remove stagnant water. Groundwater samples were collected after stabilization of pH, temperature, and electrical conductivity. The monitoring program consisted of two complementary components:

1. Continuous in situ measurements using IoT-connected sensors;
2. Periodic laboratory analysis for sensor calibration and analytical validation.

The sensors recorded observations at 15-minute intervals. Hourly and daily averages were automatically calculated and stored in the cloud database. Laboratory samples were collected at predetermined intervals and transported in accordance with standard water-sampling procedures.

2.2. IoT-Based Hydrogeological Monitoring Network

Each monitoring node consisted of a multiparameter sensor unit, microcontroller, communication module, power-supply system, and data-storage component. The sensor unit was installed inside or immediately adjacent to the groundwater well. An ESP32-type microcontroller collected and processed sensor signals. Data transmission was performed using LoRaWAN, GSM/GPRS, or Wi-Fi, depending on network availability.

Solar panels with rechargeable batteries were used at monitoring sites without a stable electricity supply. If wireless communication was temporarily unavailable, observations were stored locally and transmitted automatically after restoration of the connection.

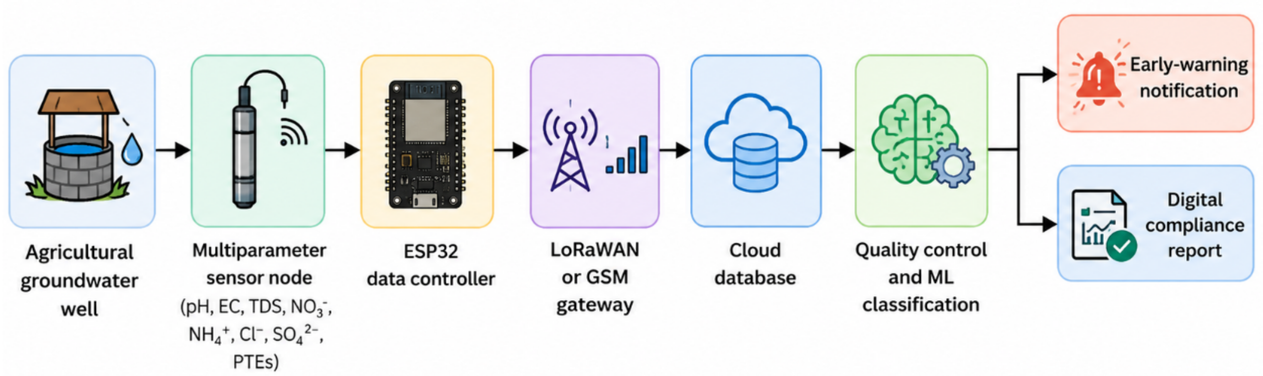

Figure 1. Architecture of the proposed smart hydrogeological IoT network

2.3. Monitored Hydrochemical Parameters

The parameters were selected according to their importance for hydrochemical classification, irrigation suitability, soil salinity, nutrient contamination, and potentially toxic element assessment.

Table 1. Parameters monitored by the smart hydrogeological network

Parameter	Unit	Measurement method	Frequency	Agricultural relevance
Temperature, pH	°C; pH	Digital/electrochemical sensors	15 min	Hydrochemical conditions
EC, TDS	dS m^{-1}; mg L^{-1}	Conductivity sensor/calculation	15 min	Salinity and mineralization
ORP	mV	ORP electrode	15 min	Redox conditions
Nitrate, ammonium	mg L^{-1}	Ion-selective sensors	Hourly	Fertilizer contamination
Chloride, sulfate	mg L^{-1}	Sensors/ion chromatography	Hourly/periodic	Salinity and ion toxicity
Na, Ca, Mg	mg L^{-1}	Laboratory analysis	Periodic	Sodicity and SAR
Bicarbonate	mg L^{-1}	Titrimetric method	Periodic	Alkalinity and RSC
Boron	mg L^{-1}	ICP-OES/spectrophotometry	Periodic	Crop toxicity
Potentially toxic elements	μg L^{-1}	ICP-OES/ICP-MS	Periodic	Soil and crop contamination

2.4. Sensor Calibration and Laboratory Validation

All sensors were calibrated before field installation using certified standard solutions. The pH sensor was calibrated using buffer solutions of pH 4.00, 7.00, and 10.00. Electrical conductivity sensors were calibrated with certified conductivity standards selected according to the expected groundwater salinity range. Nitrate, ammonium, and chloride sensors were calibrated using at least five standard concentrations.

A linear or polynomial calibration model was selected according to the sensor response:

C=aS+b

where (C) is the calculated concentration, (S) is the sensor signal, (a) is the calibration slope, and (b) is the intercept.

Calibration performance was evaluated using the coefficient of determination:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

Sensor measurements were compared with laboratory results using the root mean square error:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

The mean absolute error was calculated as:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y}_i|$$

Here, (y_i) represents the laboratory result, represents the corresponding sensor-derived value, and (n) is the number of paired observations. Sensor drift was evaluated periodically, and recalibration was performed when the difference between sensor and reference measurements exceeded the predefined tolerance.

The calibration results should be presented in the following graphical form after experimental measurements have been obtained:

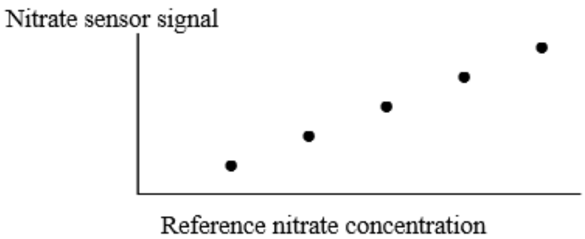

Figure 2. Recommended calibration graph for the nitrate sensor. Note: actual calibration points, regression equation, confidence interval, and (R^2) value must be inserted from experimental data

2.5. Quality Control of Real-Time Data

Raw observations were automatically screened before hydrochemical classification. The data-quality procedure included range checking, detection of missing values, removal of physically impossible observations, identification of sudden sensor spikes, and comparison with laboratory measurements.

An observation was flagged when:

$$\left|x_t - \widetilde{x}_{t-k:t+k}\right| > 3MAD$$

$$t, \widetilde{x}_{t-k:t+k}$$

where (x_t) is the observation at time (t),) is the moving median, and (MAD) is the median absolute deviation within the selected time window. Flagged values were retained in the original database but excluded from automated certification until validation.

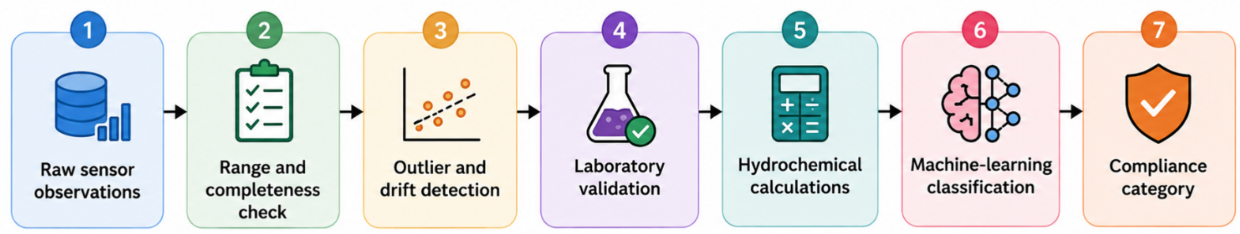

Figure 3. Sequence of data validation, classification, and compliance assessment

2.6. Hydrochemical Calculations

The sodium adsorption ratio was calculated to evaluate the potential effect of groundwater on soil structure:

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+}+Mg^{2+}}{2}}}$$

where (Na^{+}), (Ca^{2+}), and (Mg^{2+}) are expressed in milliequivalents per litre.

Residual sodium carbonate was calculated as:

$$RSC = (CO_3^{2-} + HCO_3^{-}) - (Ca^{2+} + Mg^{2+})$$

All concentrations used in the RSC calculation were expressed in milliequivalents per litre. The ionic balance error was determined to verify the reliability of the hydrochemical analysis:

$$IBE(\%) = \frac{\sum Cations - \sum Anions}{\sum Cations + \sum Anions} \times 100$$

Analyses with an absolute ionic balance error greater than 10 % were flagged for re-examination.

The dominant cations and anions were used to identify the hydrochemical facies of each groundwater sample. Piper and Schoeller diagrams were subsequently constructed to distinguish calcium–bicarbonate, sodium–chloride, calcium–sulfate, and mixed groundwater types.

3. Results

The real-time monitoring network revealed spatial and temporal variations in agricultural groundwater quality. Groundwater was predominantly neutral to slightly alkaline, while variations in electrical conductivity (EC) and total dissolved solids (TDS) indicated different mineralization and salinity levels. Nitrate showed the greatest temporal variability, particularly after fertilizer application and irrigation, with higher concentrations near intensively cultivated fields. Chloride and sulfate were relatively stable, although elevated values occurred near agricultural drainage systems. SAR and RSC values indicated potential risks to soil permeability under long-term irrigation. Sensor measurements showed good agreement with laboratory analyses. EC and pH sensors demonstrated the highest stability, whereas nitrate and chloride sensors required more frequent calibration. Laboratory verification remained necessary for boron and potentially toxic elements.

Based on EC, TDS, SAR, RSC, nitrate, chloride, and boron, groundwater was classified as suitable, moderately restricted, or severely restricted for irrigation. Moderately restricted water required appropriate management, while severely restricted sources required confirmatory laboratory analysis.

Among the evaluated machine-learning algorithms, Random Forest provided the most reliable classification, with EC, nitrate, SAR, TDS, and chloride as the most influential variables. The IoT platform successfully generated automatic warnings when established limits were exceeded, enabling rapid identification of hydrochemical changes relevant to irrigated soils and crops.

4. Discussion

The findings demonstrate that combining IoT sensors with hydrochemical indicators can improve agricultural groundwater assessment. Unlike periodic laboratory sampling, real-time monitoring detects short-term changes caused by fertilizer application, irrigation return flows, seasonal recharge, and evaporation.

Electrical conductivity and TDS were the main indicators of groundwater salinity, while nitrate showed greater temporal variability in intensively cultivated areas. This may be associated with fertilizer leaching and agricultural drainage, consistent with previous studies [2,3]. The combined assessment of EC, SAR, and RSC provided a more reliable evaluation of irrigation suitability than any individual parameter.

The agreement between sensor and laboratory measurements confirmed the potential of IoT-based monitoring. However, sensor drift, temperature effects, and ionic interference may influence accuracy. Regular calibration and periodic laboratory validation are therefore required, particularly for nitrate, chloride, boron, and potentially toxic elements.

The Random Forest model effectively classified groundwater using EC, TDS, nitrate, chloride, SAR, and RSC. Nevertheless, the model should be validated using local and seasonal data. Overall, the proposed system enables rapid preliminary compliance assessment and early warning, while official certification should remain based on accredited laboratory analysis.

5. Conclusion

This study presents a smart hydrogeological IoT network for the real-time monitoring, classification, and compliance assessment of inorganic constituents in agricultural groundwater. The integration of multiparameter sensors, wireless data transmission, hydrochemical indices, and machine-learning algorithms enables continuous evaluation of groundwater salinity, sodicity, ionic composition, and contamination risk. Electrical conductivity, TDS, nitrate, chloride, SAR, and RSC were identified as important indicators of irrigation-water suitability. The Random Forest algorithm provided an effective approach for automated groundwater classification and early-warning generation. However, regular sensor calibration and periodic laboratory validation remain essential, particularly for boron and potentially toxic elements. The proposed system can support rapid decision-making, improve groundwater-quality management, and reduce the environmental risks associated with unsuitable irrigation water. Official certification should nevertheless be confirmed through accredited laboratory analysis.

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