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Analysis of DDT and heavy metal residues in eggs of storks living in agricultural areas

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Abstract: The Oriental stork village in Yesan, Chungcheongnam-do is a representative ecological conservation area where eco-friendly agriculture and the restoration of the Oriental stork (*Ciconia boyciana*) are being implemented simultaneously. However, residual contamination by banned agricultural pesticides such as DDT and heavy metals historically used in farmland may pose potential threats to reproductive success and ecosystem stability of the stork population. QuEChERS-based extraction method was modified to enhance extraction efficiency for high-lipid and high-protein samples such as bird eggs, and its performance was validated in terms of recovery, precision, linearity, and detection limits. Heavy metals were analyzed using ICP-MS, ICP-OES, and a mercury analyzer following streamlined analytical procedures adapted from established standard protocols. Unfertilized and naturally abandoned stork eggs collected during the breeding season, as well as surrounding soil, river water, and prey organisms (frogs, loaches, and horse mackerels), were analyzed for 34 pesticides and 11 heavy metals. The persistent DDT metabolite p,p′-DDE was detected in stork eggs and at trace levels in some prey organisms, indicating potential dietary exposure through the food web. Fipronil sulfone was also detected in soil and biological samples, suggesting environmental persistence of modern insecticides. Among heavy metals, trace concentrations of As, Pb, and Hg were observed in several samples. These findings indicate that legacy pesticides and heavy metals continue to persist even in eco-friendly agricultural areas. Continuous environmental monitoring and management efforts are needed to ensure successful conservation of the Oriental stork population. This study provides baseline data for environmental safety assessment using avian egg–based biomonitoring.

Key words: Oriental stork (*Ciconia boyciana*) #1, Egg #2, DDT and p,p′-DDE #3, Fipronil sulfone #4, Heavy metals #5

1. Introduction

Hwangsae Village in Yesan-gun, Chungcheongnam-do, was established in 2009 as part of a national restoration program for the Oriental stork (*Ciconia boyciana*). Since then, eco-friendly farming practices

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have been introduced to promote habitat conservation and sustainable agricultural production. Currently, safe agricultural products, including rice, are cultivated across approximately 1.5 million m^2 of farmland managed under environmentally friendly practices. Despite these efforts, concerns remain regarding residual contamination in soil and surrounding ecosystems caused by historical pesticide use, particularly those with long environmental persistence. Continuous research is therefore necessary to assess the ecological impacts of such persistent pesticide residues. In Korea, the registration and use of pesticides with a soil half-life exceeding 180 days are restricted, and more than 98 % of currently registered pesticides are reported to have half-lives shorter than 120 days.[1] Nevertheless, p-p'-DDT (dichlorodiphenyltrichloroethane), a widely used organochlorine insecticide in the past, is known as a representative persistent organic pollutant (POP) that remains in the environment for extended periods and undergoes bioaccumulation and biomagnification through the food web.[2] DDT readily accumulates in the fatty tissues of plants and animals and is transferred to higher trophic-level predators, such as birds and mammals, where high concentrations may be detected.[3] Consequently, DDT has been associated with various neurotoxic, endocrine-disrupting, and reproductive abnormalities, leading most countries to ban its use in the 1970s (1972 in the United States; discontinued in Korea after 1979).[4] DDT is characterized by extremely slow degradation, with reported soil half-lives ranging from 15 to as long as 30 years.[5] During the 2017 “pesticide-contaminated eggs incident” in Korea, DDT was found in commercial eggs, and the contamination was attributed to impurities present in imported dicofol formulations.[6]

Moreover, major metabolites of DDT, including DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane) also exhibit high environmental stability, making their complete removal difficult and allowing continued bioaccumulation through food chains.[7] Residual DDT contamination in ecological restoration zones such as Hwangsae Village may pose potential risks to ecological function, physiological health, and reproductive success of wildlife. First, soil and water contamination may reduce microbial and benthic biodiversity, disrupting the food web and subsequently decreasing the abundance of fish, amphibians, and insects that constitute the primary diet of the Oriental stork. Second, direct exposure to DDT may induce neurological and immunological disturbances in the species. Third, accumulation of DDT and its metabolites has been recognized as a major cause of reproductive failures, including eggshell thinning and reduced hatching success.[3]

The degradation of DDT is influenced by multiple environmental factors, including soil organic matter content, pH, temperature, microbial activity, moisture, and photo degradation intensity. In soils rich in organic matter, DDT may bind strongly to organic particles, thereby delaying biodegradation. Under acidic conditions (low pH), the chemical stability of DDT increases, slowing its degradation rate, whereas high temperatures and enhanced microbial activity promote comparatively rapid break down.[8,14]

In this study, residual pesticide analysis of 34 insecticides was conducted on Oriental stork eggs collected from Hwangsae Village, and p,p'-DDE was identified as the only detectable compound. To trace the potential origin of the detected p,p'-DDE, additional environmental matrices including soil, river water, and potential prey organisms—were examined for the same target pesticides. Trace peaks of p,p'-DDE were identified in some prey organisms, while Fipronil sulfone was detected in both soil and biological samples. Based on these findings, DDT was selected as the primary target compound for detailed assessment of concentration levels, distribution characteristics, and ecological significance. In this study, the term “DDT” refers collectively to DDT and its major degradation products (p,p′-DDE and p,p′-DDD). Fipronil sulfone is known to be highly lipophilic and environmentally persistent, and previous studies have reported its potential for bioaccumulation and trophic transfer, raising concern for adverse effects in upper-level predators.[9] Therefore, in this study, fipronil and its major metabolite fipronil sulfone were included as key target compounds together with DDT. In addition, 11 toxic heavy metals (Ag, Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, and Pb) were simultaneously analyzed to comprehensively characterize contamination status and potential ecological risks in the Oriental stork habitat.

In particular, arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are well-recognized toxic metals with high potential for bioaccumulation and biomagnification in wildlife.[10] Nickel (Ni), chromium (Cr), copper (Cu), cobalt (Co), manganese (Mn), and aluminum (Al) are also widely distributed in the environment and may induce sub-lethal to lethal physiological effects under chronic exposure conditions.[11] Silver (Ag) was additionally included as an emerging contaminant of concern, given its increasing use in antimicrobial and industrial applications and the growing evidence of ecotoxicological impacts on aquatic organisms.[12] The results of this study provide evidence that historical pesticide residues may persist even in eco-friendly agricultural zones and offer important insights into how such contaminants influence local ecosystems and avian populations. These findings are expected to serve as foundational data for establishing environmental management strategies and pollution mitigation plans for ecological restoration sites.

2. Experimental

2.1. Sampling areas and methods

Sampling was conducted in the major breeding and nesting habitats of the Oriental stork (*Ciconia boyciana*). In particular, the study focused on areas surrounding the Yesan Oriental Stork Ecology Center in Yesan-gun and the release sites in Taean-gun. The geographical locations of all sampling points are shown in *Fig.* 1.

Egg samples were collected from unhatched eggs (infertile or failed to hatch eggs) found in nests within the natural release zone of Yesan Stork Village and the release sites in Taean-gun. All egg samples were obtained in compliance with relevant legal procedures and with full cooperation from the Yesan Oriental Stork Ecology Center.

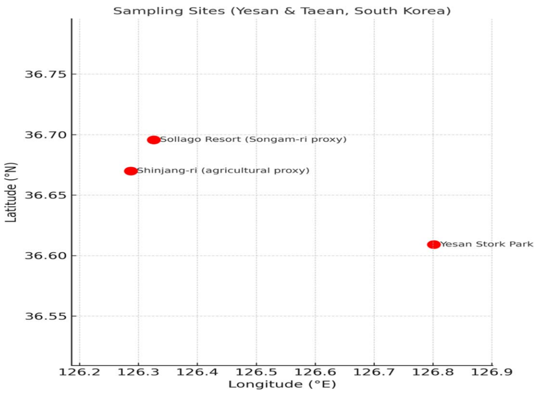

Fig. 1. Sampling area (Yesan and Taean, Republic of Korea)

Stork egg samples consisted of unfertilized or failed-to-hatch eggs collected during the spring breeding seasons of 2024 and 2025. A total of 12 eggs from Yesan and 11 eggs from Taean were collected and used for analysis. For surrounding environmental matrices, soil, water, and prey organisms were sampled in May 2025. At each study site, five replicate samples per matrix were collected and analyzed.

Soil samples were collected from three points within a 5–10 m radius around each nest.

Surface soils (15 cm) were gathered and subsequently combined and homogenized to prepare representative composite samples. River water samples were collected from nearby streams adjacent to nesting sites. To minimize interference from floating debris and organic matter, surface water samples were collected at a depth of approximately 10–20 cm using pre-cleaned high-density polyethylene (HDPE) bottles. The term "continuous sampling" refers to the gradual collection of water over a short period (approximately 3–5 min) at each site while gently moving the sampling bottle along the water flow, in order to obtain a representative composite surface water sample. For prey organism samples, horse mackerels and loaches were obtained from external feed sources used within the Yesan Stork Park. In contrast, in Taean-gun, frogs and loaches were directly collected from aquatic environments near the nesting sites. All samples including eggs, soil, river water, and prey organisms were stored under refrigerated or frozen conditions prior to analysis, and were thoroughly homogenized immediately before pretreatment.

2.2. Target compounds

The selection of target compounds for this study was based primarily on the Food Code established by the Ministry of Food and Drug Safety (MFDS) and the monitoring items designated by the Ministry of Environment for water and soil contamination.[13,14]

In addition, the ecological feeding habits of the Oriental stork including fish, amphibians, and insects were considered, and a total of 34 pesticides were selected, focusing on insecticide classes historically used at high frequencies in agricultural environments or previously reported to pose ecological risks.[15] These compounds encompass various chemical classes, including organochlorines (e.g., DDT), organophosphates (e.g., diazinon, dichlorvos), carbamates (e.g., carbaryl), pyrethroids (e.g., bifenthrin, cypermethrin), neonicotinoids (e.g., clothianidin, imidacloprid), avermectins (e.g., abamectin, ivermectin), and spinosyns (e.g., spinosad). These pesticide groups are widely detected in agricultural environments and are known to exert a range of ecological effects.[16]

Analysis of stork egg samples revealed that among the 34 target pesticides, only DDT related compounds were detected. Accordingly, additional environmental matrices including soil, river water, and prey organisms were analyzed to verify the presence of DDT and Fipronil. Both compounds have been reported globally in past and present agricultural environments, and due to their environmental persistence and biomagnification potential, they were selected as representative legacy pesticides for further evaluation.[17,18] For heavy metals, eleven elements were selected: four toxicologically critical metals that are internationally monitored due to their environmental and ecological risks (As, Cd, Hg, Pb), and seven essential trace elements (Al, Co,

Table 1. List of 34 pesticide compounds analyzed and their chemical classes and analytical instruments

Compound name	Chemical class	Half-life in field soil	Analytical Instrument	Compound name	Chemical class	Half-life in field soil	Analytical Instrument
Abamectin	Avermectin	1 day	LC−MS/MS	Fluralaner	Isoxazoline	>365 days (very stable)	LC−MS/MS
Amitraz	Amidines	0.1~3 days	LC−MS/MS	Imidacloprid	Neonicotinoid	40~997days (1~3 years)	LC−MS/MS
Bifenthrin	Pyrethroid	65~125 days (up to 8 months)	GC−MS/MS	Isofenphos	Organophosphate	7~21 days	GC−MS/MS
Carbaryl	Carbamate	7~28 days	LC−MS/MS	Ivermectin	Avermectin	7~14 days (some up to 30 days)	LC−MS/MS
Cartap	Nereistoxin analog	3~14 days	LC−MS/MS	Methamidophos	Organophosphate	2~10 days	LC−MS/MS
Chlorfenapyr	Pyrrole	14~21 days	GC−MS/MS	Methidathion	Organophosphate	15~50 days	GC−MS/MS
Clothianidin	Neonicotinoid	148~1,155 days (Up to 3 years)	LC−MS/MS	Monocrotophos	Organophosphate	3~30 days	LC−MS/MS
Cypermethrin	Pyrethroid	30~60 days	GC−MS/MS	Phorate	Organophosphate	10~60 days	LC−MS/MS
DDT	Organochlorine	2~15 years (Up to 30 years)	GC−MS/MS	Pirimiphos-ethyl Pirimiphos-methy	Organophosphate	30~180 days (4−6 months)	GC−MS/MS
Diazinon	Organophosphate	2~4 days	GC−MS/MS	Propoxur	Carbamate	7~30 days	LC−MS/MS
Dichlorvos	Organophosphate	1~7 days	LC−MS/MS	Pyridaben	Pyridazinone	20~100 days	LC−MS/MS
Etoxazole	Diphenyloxazoline	20~50days	GC−MS/MS	Spinosad	Spinosyn	~200 days	LC−MS/MS
Fenitrothion	Organophosphate	4~12 days	GC−MS/MS	Spiromesifen	Tetronic acid derivative	7~30 days	GC−MS/MS
Fenobucarb	Carbamate	2~7 days	GC−MS/MS	Sulfoxaflor	Sulfoximine	10~30 days	LC−MS/MS
Fensulfothion	Organophosphate	5~100 days	LC−MS/MS	Tetraconazole	Triazole fungicide	25~125 days	GC−MS/MS
Fipronil	Phenylpyrazole	90~200 days	LC−MS/MS	Thiocyclam	Nereistoxin analog	1~5 days	LC−MS/MS
Flufenoxuron	Benzoylurea	100~300 days	LC−MS/MS	Trichlorfon	Organophosphate	1~27 days	LC−MS/MS

Source : The half-life data of pesticides in field soil were collected from the PPDB (University of Hertfordshire, 2024), U.S. EPA Environmental Fate Database, and domestic reports from the Ministry of Food and Drug Safety (MFDS, 2023).

Table 2. List of 11 heavy metals analyzed and their environmental and toxicological characteristics

Element	Symbol	Classification	Environmental concern	Analytical Instrument
Arsenic	As	Toxic element	Carcinogenic, persistent in soil and water	ICP–MS, ICP–AES
Cadmium	Cd	Toxic element	Bioaccumulative, renal toxicity	ICP–MS, ICP–AES
Mercury	Hg	Toxic element	Volatile, neurotoxic, bioaccumulative	Mercury Analyzer
Lead	Pb	Toxic element	Persistent, hematologic toxicity	ICP–MS, ICP–AES
Chromium	Cr	Essential (Cr^{3+}), toxic (Cr^{6+})	Oxidative stress, redox toxicity	ICP–MS, ICP–AES
Nickel	Ni	Essential trace metal	High concentration causes dermatitis	ICP–MS, ICP–AES
Copper	Cu	Essential trace metal	Toxic in excess, aquatic toxicity	ICP–MS, ICP–AES
Manganese	Mn	Essential trace metal	Neurotoxicity at high levels	ICP–MS, ICP–AES
Cobalt	Co	Essential trace metal	Potentially carcinogenic at high exposure	ICP–MS, ICP–AES
Aluminum	Al	Non-essential element	Bioaccumulation, soil persistence	ICP–MS, ICP–AES
Silver	Ag	Non-essential element	Aquatic toxicity, antimicrobial interference	ICP–MS, ICP–AES

Source : The toxicological and environmental classifications of heavy metals were based on the Environmental Health Criteria (EHC) series published by the World Health Organization (WHO), the International Agency for Research on Cancer (IARC) monographs, and the U.S. ATSDR toxicological profiles (WHO, 1981–2012; IARC, 2012; ATSDR, 2020).

Cr, Cu, Mn, Ni, Ag) that may become toxic when accumulated at elevated concentrations.[19]

This selection allowed for a comprehensive evaluation of the bioaccumulation characteristics and potential ecological risks of heavy metals in stork eggs. *Table* 1 summarizes the physicochemical and environmental properties of the 34 pesticide compounds, and *Table* 2 presents the characteristics of the eleven heavy metals included in the analysis.

2.3. Standards and reagents

A total of 34 pesticide standards for screening analysis, including Abamectin and 33 additional compounds, were purchased from AccuStandard Inc. (New Haven, CT, USA), all of which had purities exceeding 99 %. Certified reference standards used for final quantitative analysis—p,p′-DDT, o,p′-DDT, p,p′-DDE, p,p′-DDD, Fipronil, and Fipronil sulfone were also obtained from AccuStandard Inc., with respective purities of 99.5 %, 97.3 %, 99.9 %, 99.1 %, 99.5 %, and 99.6 %. For heavy metals, mixed standard solutions (Ag, Al, Cd, Co, Cr, Cu, Mn, Ni, Pb; 1000 mg/L each) were obtained from Merck (Germany). A single-element arsenic standard (1000 mg/L) was supplied by AccuStandard, and mercury analysis utilized the certified reference material BCR-277R (European Commission, Belgium). High-purity reagents used for pesticide extraction and sample homogenization included L-(+)-ascorbic acid (99 %, DAEJUNG), sodium L-ascorbate (98 %, JUNSEI), magnesium sulfate anhydrous ($MgSO_4$, 99.5 %, JUNSEI), sodium chloride (NaCl, 99 %, DAEJUNG), C18 sorbent (octadecyl-bonded silica, DAEJUNG), and PSA sorbent (primary secondary amine, Agilent Technologies).

Ultrapure water (18 MΩ·cm) was produced using a Vivagen EXL-5 purification system.

Organic solvents and additives used for extraction and cleanup included acetonitrile (MeCN, 99.99 %, Burdick & Jackson), methanol (MeOH, 99.98 %, Burdick & Jackson), ammonium formate (97 %, Sigma-Aldrich), nickel(II) chloride hexahydrate (96 %, DAEJUNG), ammonium hydroxide (98 %, DAEJUNG), sulfuric acid (H_2SO_4, 98 %, DAEJUNG), L-cysteine hydrochloride anhydrous (97%, Thermo Fisher), and formic acid (98 %, Sigma-Aldrich). For heavy metal digestion and dilution, nitric acid (HNO_3, 69–71 %, Dongwoo Fine-Chem) and hydrochloric acid (HCl, 35–40 %, Dongwoo Fine-Chem) were used. All reagents were of analytical grade or higher. To minimize cross contamination, all laboratory glassware and tools were rinsed before and after use with ultrapure water (18 MΩ·cm).

2.4. Sample preparation

Sample pretreatment procedures were carried out according to the characteristics of each matrix. Egg samples were homogenized by combining the eggshell, yolk, and albumen. Soil samples were air dried and sieved through a 2 mm mesh.[20] River water samples were homogenized by gentle shaking prior to use. Prior to analysis, prey organisms were rinsed with deionized water to remove adhering soil and debris, blotted dry with clean paper towels, and weighed. Whole-body samples were then homogenized using a stainless-steel homogenizer under chilled conditions to ensure sample uniformity. The homogenized samples were stored at -20 °C until further analysis.

2.4.1. Pesticide extraction

For pesticide extraction, 5 g of each homogenized sample was transferred into a 50 mL centrifuge tube, followed by the addition of 0.5 mL of ascorbic acid mixture and 15 mL of acetonitrile containing 1 % acetic acid. The mixture was shaken for 1 min, after which 6 g of $MgSO_4$ and 1.5 g of NaOAc were added and mixed for 10 min. The mixture was centrifuged at 3,000 g for 10 min at 4 °C to obtain the crude extract. For LC–MS/MS analysis : An aliquot of 1 mL of the extract was transferred to a tube containing $MgSO_4$ (150 mg) and C18 sorbent (25 mg), vortex-mixed, and centrifuged at 13,000 g for 10 min. The resulting supernatant was filtered through a 0.2-µm PTFE membrane filter. For GC–MS/MS analysis : A 4 mL aliquot of the extract was added to a cleanup tube containing $MgSO_4$ (300 mg), PSA (100 mg), and C18 (100 mg), followed by centrifugation at 3,000 g for 10 min. The supernatant was filtered through a 0.2 µm PTFE membrane filter and used as the test solution. The pesticide extraction and cleanup procedures were based on the official analytical methods described in the Korean Food Code (Ministry of Food and Drug Safety, MFDS), which are widely applied for multiresidue pesticide analysis using a QuEChERS-based approach. Minor modifications were introduced to improve extraction efficiency and matrix cleanup for samples with high lipid and protein contents, such as avian eggs and whole-body prey organisms. Conversion of Cartap and Thiocyclam to Nereistoxin : Because Cartap and Thiocyclam are enzymatically and chemically converted to Nereistoxin during analysis, an additional chemical conversion step was included in the pretreatment. A 10-g homogenized sample was extracted with 40 mL of 2 % cysteine solution (30 mL for egg samples) for 30 min. The pH was adjusted to 4–5, followed by centrifugation at 3,500 g for 10 min. A 20 mL aliquot of the supernatant was transferred to a tube, and 1.5 mL each of 3 % $NiCl_2$ solution and NH_4OH were added. The mixture was reacted at 70 °C for 1 h. After cooling, the solution was adjusted to pH 5 with 10 N H_2SO_4, and 10 mL of hexane was added. The upper hexane layer was discarded.

The aqueous layer was then adjusted to pH 9 with 10 N NaOH, followed by the addition of 6 g NaCl and 20 mL acetonitrile. After centrifugation, the upper acetonitrile phase was filtered through a 0.45 µm PTFE membrane filter and used as the test solution. The conversion procedure of cartap and thiocyclam to nereistoxin followed the MFDS Food Code method for nereistoxin-related insecticides, with slight adjustments in sample amount and extraction volume depending on the sample matrix.

2.4.2. Heavy metal digestion

Acid digestion procedures for heavy metal analysis were conducted in accordance with the Korean Food Code and the Korean Soil Pollution Standard Methods, depending on the sample matrix. The digestion conditions were slightly simplified to improve analytical throughput while maintaining consistency with established protocols.

Table 3. Sample preparation for heavy metals (Acid digestion conditions by matrix)

Matrix	Sample amount	Acid composition	Digestion condition	Equipment	Remarks
Stork egg	1 g	HNO_3 8 mL	200°C, 30 min	Microwave (START D)	Diluted to 25 mL and filtered through 0.45 μm membrane
Soil	1 g	HCl 6 mL + HNO_3 2 mL	200°C, 60 min	Microwave (START D)	Same as above
River water	10 mL	HNO_3 8 mL	200°C, 30 min	Hotplate	Same as above
Prey organism	0.2 g	HNO_3 8 mL	200°C, 30 min	Microwave (START D)	Same as above

Table 4. Simultaneous LC-MS/MS, GC-MS/MS analysis conditions

LC-MS/MS				**GC-MS/MS**	
Ionization method	ESI positive and negative ion mode			Ionization	Electron impact (EI)
Capillary voltage	3.0 kV			EI eneragy	70 eV
Collision gas	Nitrogen(N_2)				
Column	ACQUITY UPLC BEH C18 130Å, 1.7 mm, 2.1 mm × 100 mm			Column	DB-5MS UI (30 m × 0.25 mm, 0.25 μm)
Column temp.	40°C			Injection mode	Splitless
Mobile Phase	A : Water containing 0.1 % formic acid and 5 mM ammonium formate B : Methanol containing 0.1 % formic acid and 5 mM ammonium formate			Mobile phase gas	Helium (He)
Injection volume	5 μL			Injection volume	2 μL
Flow rate	0.3 mL/min			Flow rate	1.5 mL/min
Gradient mode	Time(min)	A(%)	B(%)	Inlet temp.	280°C
	0	80	20	Ion source temp.	280°C
	0.1	80	20	Interface temp.	300°C
	14	5	95	Oven temperature	Inject the test solution at 60°C, then increase the temperature to 180°C at a rate of 20°C/min, then increase to 300 °C at a rate of 5°C/min, and maintain for 5 minutes.
	15	0	100		
	15.1	80	20		
	18	80	20		

Acid digestion procedures for each sample matrix are summarized in *Table* 3. All digested samples were cooled and diluted to a final volume of 25 mL, then filtered through a 0.45 μm PTFE membrane filter prior to analysis by ICP–MS or ICP–OES. For mercury (Hg), homogenized samples were analyzed directly using the Mercury Analyzer without any additional pretreatment.

2.5. Analytical instruments and conditions

2.5.1 Pesticide analysis

Pesticide residues were analyzed using a Waters TQ-XS LC–MS/MS system and a Shimadzu Q8050NX GC–MS/MS system. The LC–MS/MS instrument was employed for the determination of polar and semi-volatile pesticides (e.g., Fipronil, Cartap), operating in both positive and negative electrospray ionization (ESI) modes. Quantification was performed in Multiple Reaction Monitoring (MRM) mode by selecting optimized precursor product ion transitions for each compound. The GC–MS/MS system was used for the analysis of non-polar and volatile pesticides (e.g., DDTs, diazinon), operated under electron impact (EI, 70 eV) ionization. Each compound was analyzed using optimized retention times, collision energies, and ion pairs specific to the target analytes. The detailed instrumental conditions for both LC–MS/MS and GC–MS/MS analyses are summarized in *Table* 4, *Table* 5. Optimized MS/MS conditions for DDT and fipronil

LC-MS/MS								
Pesticide	R.T. (min)	Precursor ion(m/z)	Quantification transition (m/z)	D.P. (V)	C.E. (V)	Confirmatory transition(m/z)	D.P. (V)	C.E. (V)
Fipronil	11.73	437	368	60	18	290	60	25
FIpronil Sulfone	12.25	450.9	281.9	30	25	414.8	30	15

GC-MS/MS							
Pesticide	R.T. (min)	Quantitative ionpair	C.E. (V)	Quantitative ionpair	C.E. (V)	Quantitative ionpair	C.E. (V)
o,p'-DDT	15.91	235.00>165.00	27	237.00>165.00	25	235.00>199.00	15
p,p'-DDD	15.91	235.00>165.00	21	237.00>165.00	25	235.00>199.00	21
p,p'-DDE	14.55	246.00>176.00	30	248.00>176.00	30	318.00>246.00	33
p,p'-DDT	17.13	235.00>165.00	18	237.00>165.00	25	235.00>199.00	12

Table 6. Instrumental operating conditions for heavy metal analysis

ICP-MS		ICP-AES	Mercury Analyzer	
Gas used	Ar gas 99.999%	Ar gas 99.999%	Gas used	O_2 gas (99.999%)
Rf power (W)	1600	1150	Absorbance	254 nm
Coolant gas flow rate (L/min)	1.24	12	Analyze	After drying at 200°C for 60 seconds
Auxiliary gas flow rate (L/min)	1.2	0.5		
Nebulizer gas flow rate (L/min)	0.98	0.5		Decompositionat 650°C for 90 seconds
Element	Trace elements such as As, Cd, and Pb	Soil samples were analyzed for both trace metals and major elements	Element	Hg

while the optimized analytical parameters for the representative target compounds, DDT and Fipronil, are provided in *Table* 5.

2.5.2. Heavy metal analysis

Heavy metal analysis was performed using a PerkinElmer NexION 1000 ICP–MS, a Thermo Fisher iCAP 7400 ICP–AES, and a Milestone DMA-80 Mercury Analyzer. The ICP–MS system was used for the precise quantification of trace-level elements (e.g., As, Cd, Pb) at the ppb level, while the ICP–AES system was applied for the simultaneous determination of relatively high-concentration elements such as Al, Mn, and Cu. Total mercury (Hg) was measured using a DMA-80 Mercury Analyzer, which enables direct thermal decomposition analysis without chemical pretreatment. Instrumental conditions for each analytical system were optimized based on standard analytical protocols and manufacturer recommendations. The detailed operating parameters for the ICP–MS, ICP–AES, and Mercury Analyzer are summarized in *Table* 6.

2.6. Optimization of sample extraction and clean-up

Egg and prey samples from Oriental storks contain high levels of lipids and proteins, which can cause significant matrix interference and reduced sensitivity when applying conventional QuEChERS procedures.[21] To overcome this issue, a freezing-out step (-20 °C, 1 h) was incorporated to precipitate and remove lipids prior to cleanup. The clarified supernatant was then subjected to sorbent-based cleanup.[22] For samples with high lipid content, additional C18 sorbent was used to adsorb residual lipophilic components. The same cleanup strategy was applied to protein-rich prey samples to minimize matrix-derived interferences. This optimized procedure significantly improved

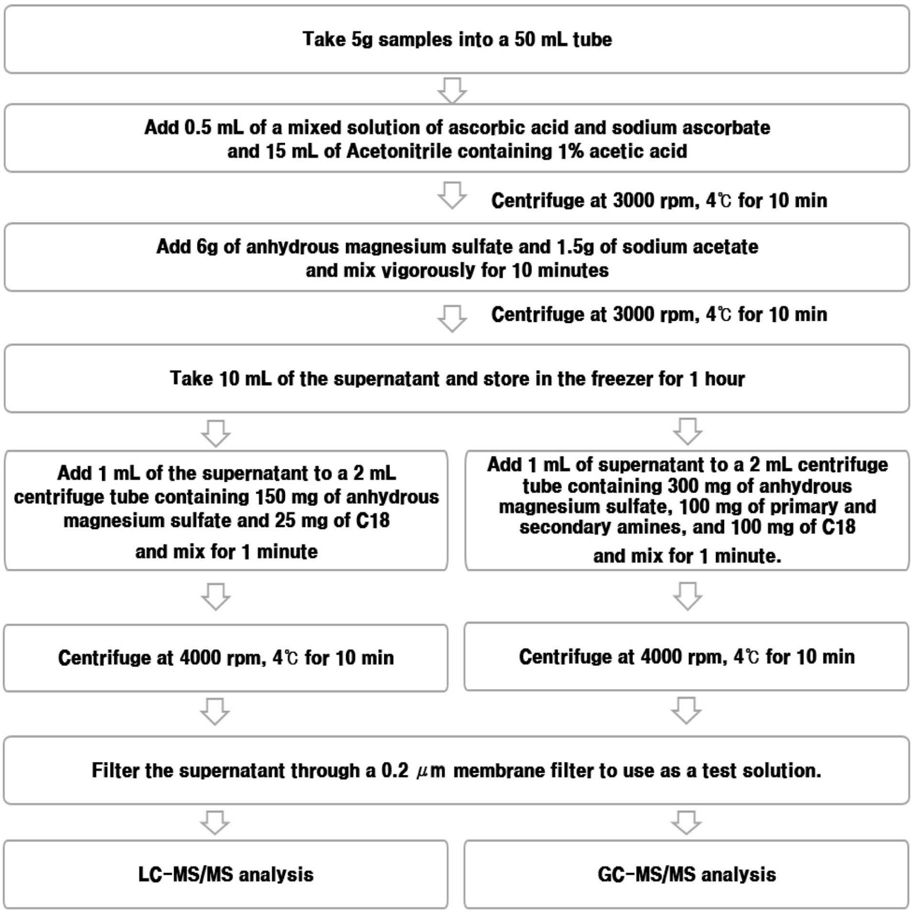

Fig. 2. LC-MS/MS, GC-MS/MS sample preparation.

matrix removal efficiency, resulting in enhanced recovery and analytical sensitivity. An overview of the entire pretreatment workflow is presented in *Fig.* 2.

2.7. Method validation

In this study, method validation was performed for representative pesticide residues, including DDT and its metabolites as well as Fipronil and its metabolites, along with 11 heavy metals (Ag, Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb). Egg and soil samples were used as representative matrices. Validation parameters included linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, and precision. The LOD and LOQ values were calculated according to the procedures described in ICH Q2(R2): Validation of Analytical Procedures (2023)[23] using the following equations:

$$\mathrm{LOD} = 3.3 \times \delta/b \quad \mathrm{LOQ} = 10 \times \delta/b$$

δ: The standard deviation of the response
b : The slope of the calibration curve

Linearity was evaluated using the coefficient of determination (R^2) obtained from calibration curves. Recovery tests were conducted at spiking levels equivalent to 5× and 50× the LOD, with triplicate analyses performed for each concentration. Recovery was evaluated by comparing analyte concentrations measured in spiked samples with those in unspiked samples. To account for native background levels, recoveries were calculated by subtracting the concentration measured in the corresponding blank or non-spiked matrix. Spiking was performed prior to extraction, and quantification was conducted using matrix-matched calibration curves.

Table 7. Detection of residual pesticides through screening

Chemical class	Representative compounds	Detected compounds	Detected matrix	Remark
Organophosphates	Phorate (including metabolites), Dichlorvos, Diazinon, Fensulfothion, Fenitrothion, Methamidophos, Monocrotophos, Isofenphos, Trichlorfon, Pirimiphos-methyl	-	-	-
Carbamates	Carbaryl, Propoxur, Fenobucarb	-	-	-
Pyrethroids	Bifenthrin, Cypermethrin	-	-	-
Neonicotinoids	Imidacloprid (including metabolites), Clothianidin, Sulfoxaflor	-	-	-
Phenylpyrazoles	Fipronil (including metabolites)	Fipronil sulfone	Soil Frog, loach	Taean : soil, Frog, loach
Macrocyclic lactones	Abamectin, Ivermectin, Spinosyn A, D	-	-	-
Nereistoxin analogues	Nereistoxine, Cartap, Thiocyclam	-	-	-
Organochlorines	DDT (including metabolites)	p,p´-DDE	Stork eggs sardines	Detected in all stork eggs and sardine samples collected in Yesan park area
Amidines	Amitraz	-	-	-
Tetrazoles	Tetraconazole	-	-	-
Others	Flufenoxuron, Etoxazole, Methidathion, Spiromesifen, Fluralaner, Pyridaben, Chlorfenapyr	-	-	-

Recovery (%) was calculated as follows: (Measured concentration in spiked sample − native concentration) / spiked concentration × 100.

These procedures conform to the requirements of Codex, SANTE (2023), and the Korean Ministry of Food and Drug Safety (MFDS) guidelines. Therefore, the validated analytical methods used in this study demonstrate sufficient reliability for assessing organic and inorganic contaminants in Oriental stork eggs and associated ecological matrices.

3. Results and Discussion

3.1. Screening results of pesticide residues

A total of 34 pesticides were screened in stork eggs, soil, river water, and prey organisms (horse mackerels, frog, and loaches). Most target pesticides were not detected; however, p,p′-DDE was identified in stork egg samples. p,p′-DDE is a well-known major metabolite of DDT. Additional analyses revealed that p,p′-DDE was not detected in soil or river water samples but was observed at trace levels in sardine samples.

Furthermore, Fipronil sulfone was detected in some soil samples (Taean region) and in prey organisms (mudfish and frogs), whereas it was not detected in soil samples collected from the Yesan region. Detection results for each sample type are summarized in *Table* 7.

3.2. Validation results of the analysis method

To ensure the reliability of the analytical results for Oriental stork eggs, soil, river water, and prey organisms, the analytical methods for pesticide residues (DDT and Fipronil) and 11 heavy metals (Ag, Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb) were validated using egg and soil as representative matrices. The validation parameters included linearity, limits of detection (LOD), limits of quantification (LOQ), recovery, and precision, and the results are summarized in *Tables* 8 and 9.

The calibration curves for DDT and Fipronil exhibited excellent linearity, with coefficients of determination (R^2) exceeding 0.998. The LOD values ranged from 0.0001 to 0.0007 mg/kg, and the LOQ values ranged from 0.0004 to 0.0022 mg/kg. Recoveries were within 71.7–101.94 %, and the relative standard deviations (RSDs) were below 5 %, meeting the criteria of

Table 8. Validation results of eggs

Instrument	Analysis	Recovery±RSD(%) (n=5)		Linearity (5point) (R^2)	LOD (mg/kg)	LOQ (mg/kg)
		Low	High			
LC-MS/MS	FIpronil	83.94 ± 1.79	87.88 ± 0.82	0.9982	0.0005	0.0015
LC-MS/MS	FIpronil Sulfone	86.12 ± 0.61	96.61 ± 0.88	0.9985	0.0002	0.0005
GC-MS/MS	o,p'-DDT	75.20 ± 1.60	77.56 ± 0.72	0.9999	0.0002	0.0006
GC-MS/MS	p,p'-DDD	74.10 ± 0.31	79.40 ± 1.37	0.9997	0.0007	0.0020
GC-MS/MS	p,p'-DDE	75.58 ± 3.00	75.40 ± 0.36	0.9998	0.0003	0.0010
GC-MS/MS	p,p'-DDT	71.71 ± 1.57	77.74 ± 0.19	0.9997	0.0006	0.0017
ICP-AES	Ag	93.41 ± 0.74	95.74 ± 0.92	0.9999	0.0015	0.0047
ICP-AES	Al	97.54 ± 3.30	98.67 ± 2.27	0.9994	0.0016	0.0048
ICP-MS	As	93.83 ± 3.19	95.11 ± 2.85	0.9999	0.0001	0.0002
ICP-MS	Cd	102.26 ± 2.10	94.57 ± 1.20	0.9999	0.0001	0.0001
ICP-AES	Co	102.16 ± 1.27	100.24 ± 1.65	0.9998	0.0004	0.0014
ICP-AES	Cr	109.55 ± 2.12	107.95 ± 1.94	0.9999	0.0010	0.0030
ICP-AES	Cu	101.82 ± 1.21	99.98 ± 0.86	0.9991	0.0012	0.0037
ICP-AES	Mn	108.91 ± 2.05	106.37 ± 1.85	0.9999	0.0001	0.0004
ICP-AES	Ni	107.05 ± 1.38	105.37 ± 1.78	0.9999	0.0008	0.0024
ICP-MS	Pb	98.33 ± 2.26	96.46 ± 3.50	0.9999	0.0002	0.0005
Mercury Analyzer	Hg	104.71 ± 2.18	96.21 ± 1.50	0.9998	0.00011	0.00035

Table 9. Validation results of soils

Instrument	Analysis	Recovery±RSD(%) (n=5)		Linearity (R^2)	LOD (mg/kg)	LOQ (mg/kg)
		Low	High			
LC-MS/MS	FIpronil	101.94 ± 1.28	101.41 ± 0.76	0.9995	0.0004	0.0014
LC-MS/MS	FIpronil Sulfone	100.45 ± 0.41	96.62 ± 0.94	0.9989	0.0001	0.0004
GC-MS/MS	o,p'-DDT	83.28 ± 0.35	81.51 ± 1.02	0.9990	0.0004	0.0012
GC-MS/MS	p,p'-DDD	80.50 ± 1.48	78.36 ± 0.85	0.9987	0.0004	0.0013
GC-MS/MS	p,p'-DDE	78.71 ± 1.94	79.68 ± 0.30	0.9990	0.0007	0.0022
GC-MS/MS	p,p'-DDT	77.82 ± 1.63	83.52 ± 0.34	0.9981	0.0004	0.0011
ICP-AES	Ag	95.67 ± 1.32	101.36 ± 0.60	0.9999	0.0024	0.0073
ICP-AES	Al	93.35 ± 2.81	94.44 ± 1.74	0.9994	0.0567	0.1718
ICP-AES	As	95.05 ± 0.94	97.59 ± 2.26	0.9984	0.0800	0.2500
ICP-AES	Cd	106.06 ± 3.89	102.49 ± 3.41	0.9997	0.0079	0.0236
ICP-AES	Co	96.18 ± 1.79	99.09 ± 1.13	0.9998	0.0004	0.0014
ICP-AES	Cr	95.00 ± 3.21	102.66 ± 1.25	0.9999	0.0076	0.0232
ICP-AES	Cu	95.34 ± 2.37	99.04 ± 0.73	0.9991	0.0012	0.0037
ICP-AES	Mn	99.67 ± 1.86	95.28 ± 0.31	0.9999	0.0001	0.0004
ICP-AES	Ni	106.71 ± 2.28	107.26 ± 1.15	0.9999	0.0008	0.0024
ICP-AES	Pb	98.18 ± 3.10	97.37 ± 2.54	0.9999	0.0600	0.1900
Mercury Analyzer	Hg	90.06 ± 2.78	89.44 ± 0.58	0.9998	0.00001	0.00004

international guidelines (70–120 % recovery, RSD ≤ 20 %).[24] For the 11 heavy metals, recoveries ranged from 89.4–109.5 %, with RSD values ≤ 5 %, confirming high accuracy and precision across all analytes. All analytes demonstrated RSD values below 5 %, indicating excellent repeatability and reproducibility of the analytical method. These validation results confirm that the method developed in this study is robust and suitable for the reliable quantification of trace-level pesticide residues and heavy metals in diverse environmental matrices associated with the Oriental stork ecosystem.

3.3. Pesticide residue analysis results (DDT and fipronil)

DDT, Fipronil, and their metabolites were analyzed in stork eggs, soil, river water, and prey organisms (sardine, frog, and mudfish), and the results are summarized in *Table* 7.

In the stork egg samples, p,p′-DDE, the major metabolite of DDT, was detected. A low-level p,p′-DDE peak was also observed in sardine samples among the prey organisms. Fipronil and its metabolites were not detected in egg samples; however, Fipronil sulfone was detected in soil and certain prey organisms. Among the soil samples, those collected from the Yesan region (near the breeding facility) showed no detection, whereas soil samples from the Taean region (near wild release sites) showed the presence of Fipronil sulfone.

3.4. Heavy metal analysis results

Eleven heavy metals were analyzed in stork eggs, soil, river water, frogs, mudfish, and horse mackerel. In stork egg and river water samples, most heavy metals were either not detected or present at trace levels.

In soil samples, aluminum (Al) and manganese (Mn) were detected at comparatively higher concentrations, whereas all other metals were present at levels below 20 mg/kg. Frogs and mudfish exhibited detectable levels of multiple heavy metals, with Al and Mn occurring at higher concentrations than other elements, showing a distribution pattern similar to that observed in soil samples. In commercially available mudfish and horse mackerel, trace amounts of arsenic (As) and mercury (Hg) were detected.

The concentrations of heavy metals measured in soil samples were within the range of natural background levels reported for Korean soils,[25] and none exceeded the soil contamination concern standards specified under the Korean Soil Environment Conservation

Table 10. Pesticide analysis results (p,p′-DDE, Fipronil-sulfone)

Sample classification		Collection area	n	p,p´-DDE (mg/kg)	Fipronil-sulfone (mg/kg)
Stork egg		Yesan	12	0.015–0.200	ND
		Taean-1	6	0.012–0.035	ND
		Taean-2	5	0.010–0.049	ND
Soil		Yesan	5	ND	ND
		Taean-1	5	ND	0.002–0.016
		Taean-2	5	ND	0.004–0.006
River water		Yesan	5	ND	ND
		Taean-1	5	ND	ND
		Taean-2	5	ND	ND
Food creature	Frog	Taean-1 (wild)	5	ND	0.037–0.062
		Taean-2 (wild)	5	ND	0.028–0.043
	Loach	Taean-1 (wild)	5	ND	0.005–0.008
		Taean-2 (wild)	5	ND	0.008–0.010
		Yesan (externalsupply)	5	ND	ND
	Pompano	Yesan (externalsupply)	5	0.001–0.004	ND

*ND: not detected, defined as concentrations below the method detection limit (LOD).

Table 11. Heavy metal concentrations in stork eggs

Main detected elements	Median (Range), mg/kg		
	Yesan (n=12)	Taean-1 (n=6)	Taean-2 (n=5)
Al	2.41(0.09–4.73)	5.72(0.47–10.97)	2.27(0.19–4.35)
As	0.065(0.03–0.10)	0.04(0.03–0.05)	0.025(0.02–0.03)
Cr	ND(≤0.07)	ND(≤0.06)	ND(≤0.06)
Cu	1.01(0.77–1.24)	0.68(0.76–1.20)	1.04(0.86–1.22)
Hg	0.06(0.05–0.07)	0.06(0.05–0.07)	0.07(0.02–0.12)
Mn	0.43(0.27–0.59)	0.43(0.27–0.590	0.81(0.21–1.41)
Pb	ND(≤0.01)	ND(≤0.01)	ND(≤0.01)

Table 12. Heavy metal concentrations in soil

Main detected elements	Median (Range), mg/kg		
	Yesan (n=5)	Taean-1 (n=5)	Taean-2 (n=5)
Al	14954.23(13384.21–16524.25)	17806.57(12370.57–23242.88)	16342.47(12940.38–19744.56)
As	ND(≤0.2)	5.36(3.75–6.96)	5.11(3.35–6.87)
Co	3.60(2.55–4.65)	8.24(7.33–9.14)	7.61(4.32–10.89)
Cr	14.54(11.85–17.23)	38.57(31.61–45.53)	33.46(14.35–52.57)
Cu	3.69(2.18-5.19)	9.95(5.26–14.63)	8.12(4.44–11.79)
Hg	ND(≤0.01)	ND(≤0.01)	ND(≤0.02)
Mn	409.58(235.43–583.73)	434.93(336.85–533.01)	451.56(145.73–757.39)
Ni	13.98(12.53–15.43)	3.42(0.89–5.95)	9.66(1.78–17.54)
Pb	15.85(11.23–20.47)	16.40(14.88–17.95)	17.49(13.21–21.77)

Table 13. Heavy metal concentrations in aquatic organisms and river water

sample		Main detected elements, Median (Range) mg/kg						
		Al	As	Cr	Cu	Hg	Mn	Pb
River water	Yesan (n=5) Taean-1 (n=5) Taean-2 (n=5)	ND(≤0.06)	ND(≤0.01)	ND	ND	ND	ND	ND
Frog	Taean-1 (n=5), wild	21.16 (15.24–27.08)	0.14 (0.07–0.21)	0.27 (0.19–0.34)	0.58 (0.35–0.81)	ND(≤0.02)	15.24 (7.20–23.28)	0.16 (0.05–0.27)
	Taean-2 (n=5), wild	16.04 (11.87–20.20)	0.15 (0.11–0.18)	0.26 (0.12–0.39)	1.90 (0.89–2.91)	0.04 (0.02–0.05)	11.15 (8.09–14.21)	0.13 (0.08–0.18)
Loach	Yesan (n=5) externalsupply	ND	0.35 (0.22–0.47)	0.22 (0.11–0.32)	0.48 (0.35–0.61)	ND	0.77 (0.09–1.45)	ND(≤0.01)
	Taean-1 (n=5), wild	75.43 (35.62–115.23)	0.26 (0.15–0.36)	0.45 (0.24–0.65)	0.79 (0.65–0.92)	ND(≤0.02)	51.48 (41.52–58.44)	0.14 (0.08–0.19)
	Taean-2 (n=5), wild	94.88 (58.62–131.14)	0.35 (0.25–0.45)	0.61 (0.38–0.84)	0.72 (0.58–0.85)	ND(≤0.02)	48.79 (36.58–61.00)	0.21 (0.11–0.31)
Horse mackerels	Yesan (n=5) externalsupply	ND	0.72 (0.46–0.97)	ND	ND	ND(≤0.01)	ND	ND

Act. Detailed analytical results are presented in *Table* 11–13.

3.5. Discussion

The contamination characteristics of soil, river water, and prey organisms were compared with those of stork eggs to evaluate potential ecological exposure pathways.

Among the target pesticides, p,p′-DDE was the only compound detected in stork eggs. Given the persistent and lipophilic nature of this metabolite, its presence may be associated with historical maternal exposure or indirect transfer through externally supplied prey, rather than reflecting current local environmental conditions. The detection of p,p′-DDE in horse mackerel supports this possibility; however, the available data are insufficient to establish a definitive exposure pathway.

Fipronil sulfone was not detected in stork eggs but was identified in soil and certain prey organisms collected from the Taean region. This distribution pattern may indicate regional differences in pesticide use history or agricultural management practices, as well as the existence of localized ecological pathways linking soil, aquatic environments, and amphibians. For heavy metals, relatively higher concentrations of Al, Mn, Cr, and Cu were observed in soil samples, while frogs and loaches showed multiple detectable metals, suggesting their potential sensitivity as bioindicator species. In contrast, most heavy metals in stork eggs were either not detected or present at trace levels, which may imply limited maternal transfer into eggs under the studied conditions. The detection of As and Hg in externally supplied prey further suggests that off-site contamination sources could influence exposure patterns within the stork population.

Overall, stork eggs in this study should be interpreted not as direct indicators of contamination in a single environmental compartment, but rather as integrated ecological markers that may reflect a combination of maternal body burden, food-web structure, external prey inputs, and region-specific environmental factors. Further studies incorporating lipid normalization, site-specific comparisons, and dietary source tracking are required to test these hypotheses.

4. Conclusions

This study evaluated the ecological indicator potential of stork (*Ciconia boyciana*) eggs by analyzing residual pesticides and heavy metals in eggs, soil, river water, and preyorganisms. Among the target pesticides, only p,p′-DDE was detected in stork eggs, which suggests long-term maternal exposure or indirect transfer through externally supplied feed rather than contamination from the current local environment. Fipronil sulfone was detected exclusively in soil and certain prey organisms from the Taean region, reflecting regional differences in pesticide use history and environmental management practices.

For heavy metals, various elements were detected in soil, amphibians, and fish however, most metals were either not detected or present only at trace levels in stork eggs. This indicates that physiological regulation in adult birds limits the transfer of heavy metals to eggs. The occurrence of As and Hg in externally supplied prey further highlights the potential influence of off site contamination sources on exposure patterns in the stork population. Taken together, these findings demonstrate that stork eggs do not represent contamination in a single environmental medium but rather serve as integrated ecological indicators reflecting maternal exposure history, food web structure, external feed inputs, and region specific environmental characteristics. Therefore, interpreting egg based contamination signals requires an integrative approach incorporating multi media analyses of soil, water, and prey organisms.

Future studies should include

(1) long-term monitoring of contaminant accumulation in adult storks,

(2) periodic evaluation of external feed sources and their input pathways, and

(3) continued assessment of soil- and water-based contamination trends, to more accurately characterize exposure dynamics and habitat quality.

Overall, this study provides foundational scientific evidence for the conservation of this endangered species and for the development of management strategies in eco friendly agricultural landscapes. The use of stork eggs as a monitoring tool offers meaningful potential for long-term ecotoxicological surveillance and conservation policy planning.

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