ECRONICON OPEN ACCESS

EC VETERINARY SCIENCE
Research Article

Human-Dog Communication as a Driver of Physiological Change: Evidence from Hair Tissue Mineral Analysis

Sasha Riess[1]* and Muhammad Ameer Hamza[2]

[1]*Pure Love & Harmony Institute, 1003 Enterprise Place, Ste 100 Arlington Texas, United States*

[2]*Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan*

***Corresponding Author:** Sasha Riess, Pure Love & Harmony Institute, 1003 Enterprise Place, Ste 100 Arlington Texas, United States.

Received: September 08, 2026; **Published:** September 29, 2026

Abstract

Human-dog communication is known to influence affect and neuroendocrine function, its impact on slower-moving physiological domains like mineral homeostasis remains unclear. This single-arm pre-post study tested whether a structured, communication-focused intervention (the Pure Love and Harmony [PLH] Formula) could alter hair tissue mineral analysis (HTMA) profiles in companion dogs (Canis lupus familiaris) (*n* = 22) without dietary or supplement changes. Standardized hair sampling and inductively coupled plasma mass spectrometry were performed at baseline and 12 weeks. Primary outcomes included stress-linked electrolytes and ratios (Na, K; Na/K, Na/Mg, Ca/Mg), potentially toxic elements (Hg, Pb, Al), and immune-relevant trace-element indices (Cu/Zn, Fe/Cu). Following the intervention, previously reported behavioral problems were substantially reduced or no longer observed. These visible improvements were accompanied by measurable shifts in mineral levels and ratios toward reference ranges, providing objective physiological support for the observed behavioral changes. Dogs exhibited significant reductions in stress-related ratios, including Na/K and Na/Mg, together with normalization of Ca/Mg. Toxic mercury (Hg) levels also decreased significantly, while the immune-related Cu/Zn ratio shifted favorably toward reference ranges. Importantly, these changes occurred in the absence of nutritional modifications, suggesting that improved owner-dog communication may reduce allostatic load and support mineral homeostasis. These findings extend human-animal interaction research beyond acute hormonal markers by introducing a chronic, integrative biomarker perspective and identifying everyday communication as a potentially low-cost approach to supporting canine welfare. Study limitations include the modest sample size and absence of a control group; future controlled trials are warranted.

Keywords: *Human-Dog Interaction; Communication; Hair Tissue Mineral Analysis; Electrolytes; Toxic Elements; Trace-Element Ratios; Canine Welfare*

Introduction

Human-dog relationships are among the most intensively studied human-animal bonds, with consistent evidence that affiliative contact can modulate neuroendocrine activity and affective state in both partners. Brief, positive interactions elevate oxytocin, a neuropeptide implicated in social bonding, and can alter cortisol dynamics in owners and dogs [1,2]. Mutual gaze between dogs and owners even triggers a positive oxytocin feedback loop, suggesting that everyday communicative behaviors—eye contact, prosody, touch—are not merely epiphenomenal but biologically active [3]. Beyond acute changes, longer-term covariation in stress physiology has been reported: human and canine hair cortisol levels correlate across seasons, implying that the dyad's relationship quality can shape chronic hypothalamic-pituitary-adrenal (HPA) activity over time [4]. In educational and wellbeing contexts, brief structured contact with companion animals

reduces perceived stress and dampens cortisol output [5]. Together, these studies position human-dog communication as a plausible driver of systemic physiological change.

While most work has operationalized "physiological change" via hormones, heart-rate variability, or behavior, far less is known about how relationship-centered interventions might influence mineral physiology—for example, stress-sensitive electrolytes and immune-relevant trace-element ratios. Hair tissue mineral analysis (HTMA) offers a non-invasive window on longer-term element status and exposure, complementing snapshot measures in blood or saliva. In dogs, hair concentrations capture multi-week to multi-month integration of both essential elements (e.g. Ca, Mg, Zn, Cu) and toxicants (e.g. Hg, Pb, Al), and show meaningful associations with blood for metals such as mercury and lead [6-8]. Importantly, HTMA validity depends on rigorous protocols because pre-treatments can shift measured values—especially for Na and K—if exogenous decontamination also removes endogenous ions [9]. When these methodological issues are addressed, HTMA can sensitively detect diet- and dog-specific differences and has begun to reveal links between trace-element patterns and clinical states [6,8].

Researchers have established fundamental scientific principles which show that communication-based alterations lead to measurable changes in mineral distribution patterns. Interactions that promote social bonding between individuals create a safer environment which leads to reduced HPA and sympathetic outflow. The body responds to ongoing stress by activating mineralocorticoid pathways which control sodium retention and potassium excretion through aldosterone [10,11]. The ability of dog owners to communicate better with their dogs will create better stress management through improved sodium and potassium balance which results in altered electrolyte handling. Trace-element relationships demonstrate low-grade inflammation and oxidative stress while the copper-to-zinc Cu/Zn ratio serves as an immune/inflammatory indicator which varies among different canine clinical populations [8]. The process of communication leads to reduced allostatic load which results in healthier Cu/Zn balance that will develop through time.

The present study tests this hypothesis directly. We evaluate whether a structured, communication-focused behavioral intervention—here termed the Pure Love and Harmony (PLH) Formula, designed to increase predictability, clarity, and affiliative signaling in routine owner-dog interactions—can, without any changes to diet or supplementation, produce measurable changes in canine HTMA profiles. Specifically, we ask whether (i) stress-related mineral indices (e.g. Na, K and ratios involving Ca, Mg, Na, K) move toward normative ranges; (ii) potentially toxic elements (e.g. Hg, Al, Pb) decrease; and (iii) immune-relevant trace-element ratios (e.g. Cu/Zn) shift in directions consistent with reduced inflammatory tone. By linking relationship quality to mineral physiology, this work extends the human-dog communication literature beyond hormones and behavior to a chronic, integrative biomarker, and explores an economical welfare lever—communication itself—that, if effective, could scale across households and clinical settings.

Materials and Methods

Study design and reporting

We conducted a prospective, single-arm, pre-post study in companion dogs to test whether a standardized, interaction-focused intervention program (Pure Love and Harmony; PLH) is associated with changes in hair tissue mineral analysis (HTMA) indices over a 12-week interval without dietary or supplement modification.

Participants and recruitment

Pet dogs owned by individuals were recruited through community online advertisements.

Inclusion criteria were:

- Age ≥ 12 months.
- Clinically healthy as per owner report and veterinary records within the past 12 months.

- Stable diet for ≥ 8 weeks prior to enrollment, with agreement to maintain diet and supplements throughout follow-up.
- Dogs had one or more of following behavioral problems leash pulling, aggression toward animals of humans, excessive barking, home soiling, feeding difficulties, destructive chewing, separation anxiety, fear of loud noises, unwanted jumping on a people.
- Tolerance of gentle handling for hair sampling.

Exclusion criteria were:

- Pregnancy or lactation.
- Systemic illness under active treatment.
- Recent (past 8 weeks) coat dyeing or bleaching.

The target sample size was ≥ 30 dyads to provide ≥ 80% power to detect a medium within-subject effect (standardized mean change ≈ 0.5) in primary ratios. Final sample size and any deviations from the plan are reported in the results section.

Owner-dog interaction intervention (PLH program)

Owners were trained to implement the PLH program, which comprised four modules designed to increase predictability, role clarity, and secure-base attachment of dogs toward their owners, so it primary point was to establish change of the owners behavior toward their dogs in everyday contexts. Delivery consisted of a 60-minute onboarding zoom session, plus four 30-minute weekly check-ins (group or individual, if needed) during the active phase. A detailed manual and daily checklists were provided, and fidelity was monitored as described below. Owners were instructed not to alter diet, supplements, or routine medications.

HTMA: Sample preparation and ICP-MS analysis

Approximately 80-100 mg of hair was digested with 4 mL HNO_3 at 85°C for 8h and brought to a final volume of 50 mL. Reference materials included Human Hair NCS GBW09101b and an in-house pooled canine hair standard (three 10g batches: black, white, brown), analyzed with each batch for quality control. Elemental quantification was performed on a ThermoFisher Scientific iCAP TQ ICP-MS, using He collision mode and O_2 reaction mode as appropriate for the analyze.

Reference ranges were derived from laboratory-provided canine HTMA intervals and cross-checked with published canine population data [6,8].

Feeding time structure

Feeding began with the owner taking a small bite of food (such as chocolate or another item) to signal the start of the meal. The act of chewing releases saliva, which dogs can smell, marking the beginning of feeding time. The dog's meal was then placed in the bowl and set on the ground, after which the owner stepped away, allowing the dog to eat without interruption. As soon as the dog left the bowl, the owner picked it up and feeding time ended, regardless of whether food remained. The same pattern was followed at every meal. Feeding followed this standardized ritual designed to make the sequence of events consistent and predictable while avoiding dependence on a precise clock-based feeding time. The ritual established predictability of process and created a safe space in which the dog could freely choose to eat. Meals were intentionally not provided at the same time each day. Instead, feeding times varied while the sequence of the feeding ritual remained consistent. This was intended to shift predictability away from a specific time of day and toward the owner's actions and the established sequence of events. In this way, the dog could learn what to expect from the feeding process without becoming dependent on a rigid clock-based routine. The owner established themselves as the constant provider of food and protection which made the dog see them as its principal attachment figure who provided safe support.

Threshold routines ("Unity")

During departures and reunions, owners were instructed to maintain a calm and neutral demeanor. When leaving or entering the home, they refrained from initiating verbal communication, direct eye contact, physical contact, or other forms of social engagement with the dog. Upon returning home, owners entered calmly and withheld social interaction until the dog had returned to a calmer behavioral state. Owners were instructed to ignore heightened excitement, attention-seeking behavior, or elevated arousal associated with reunion. The purpose of this procedure was to reduce the emotional intensity and unpredictability associated with departures and reunions and to establish these events as routine and predictable transitions. Repeated use of the same calm and consistent pattern was intended to provide the dog with a stable behavioral reference during periods of separation and reunion. Within the PLH methodology, this procedure was designed to support predictability, emotional regulation, and relational security by establishing the owner as a consistent and reliable presence across repeated departures and returns.

Walking reset and alignment

During days 1-10, no long outdoor walks were undertaken. Dogs were taken out solely for toileting. Owners conducted 2-3 daily sessions (5-7 minutes each) indoors or in the backyard to train calm heel walk and attention, both on and off leash, emphasizing following behavior and slack-leash movement. Thereafter, neighborhood walks resumed with the owner setting pace, direction, and duration. Scanning and lead-taking behaviors were interrupted and redirected to heel and attention.

Structured affiliation ("Play")

Owners conducted two to three daily sessions (5-7 minutes each) of low-arousal affiliative interaction (gentle touch, calm petting). Sessions were always owner-initiated and owner-terminated. Toy use was limited to one session per week or less, and play was stopped before escalation (e.g. vocalization, jumping, mouthing).

Owners were instructed to minimize verbal communication with their dogs, using only brief, structured phrases when necessary. They were also guided to reduce anthropomorphic behaviors as much as possible throughout the study.

Consolidation phase

Weeks 5-12 consisted of a consolidation phase, during which all modules were maintained at the same frequencies without additional training content.

Fidelity and adherence

Owners completed daily checklists (presence/absence per module; duration in minutes) and a weekly reflection (free text plus Likert-type items). At weeks 2 and 4, owners submitted two 10-minute smartphone videos (walking practice; threshold routine). Two coders, blinded to time point and hypotheses, scored videos using a 0-2 scale on pre-specified behaviors (owner initiation/termination, dog position relative to owner, leash tension, latency to settle, etc.). An adherence index (0-100) combined checklist completion (60%), video scores (30%), and missed-session penalties (10%). Pre-specified sensitivity analyses examined outcomes by adherence tertiles.

Timeline

After enrollment and baseline assessments, owners observed a 7-day run-in (to stabilize routines and complete baseline logs), followed by a 4-week active PLH phase and an 8-week consolidation phase. Hair samples were collected at baseline (T0) and 12 weeks (T1). No interim HTMA was performed to avoid repeated sampling of the same coat region.

Statistical analysis

All analyses were performed in IBM SPSS Statistics v29.0 (IBM Corp., Armonk, NY). Elemental concentrations and ratios exhibited positive skew; therefore, primary modeling used natural-log-transformed outcomes (e.g. LN_NaK = LN(Na/K)), with estimates back-transformed and presented as geometric mean ratios (GMRs) with 95% confidence intervals.

Primary endpoints: For log(Na/K), log(Na/Mg), and log(Ca/Mg), we fit linear mixed-effects models using SPSS MIXED with time (T0 vs. T1) as a fixed effect and dog ID as a random intercept. Where sample size permitted, pre specified covariates (age, sex/neuter, size class, coat type, living context, and season/month) were added as fixed effects (≤1 covariate per ~10 dogs). Denominator degrees of freedom used the Satterthwaite method. Effects are reported as GMRs (Exp[B]) and standardized mean change (Hedges' *g* for paired data), the latter computed from the model-based marginal means using SPSS syntax.

Secondary endpoints: The same MIXED framework was applied to secondary ratios (Ca/K, Ca/P, Cu/Zn, Fe/Cu, Zn/Cd, Zn/Hg) and to log-concentrations of Hg, Pb, and Al. If residual diagnostics indicated substantial non-normality/heteroscedasticity, we re-estimated models with GENLIN assuming a gamma distribution with log link as a sensitivity check and compared inferences.

Multiple comparisons: For families of related outcomes (primary set; secondary ratios; toxic elements), we controlled the false discovery rate at q = 0.05 using the Benjamini-Hochberg procedure implemented via SPSS syntax (ranking raw *p* values within each family and computing adjusted critical values). Both raw and FDR-adjusted *p* values are reported.

Robustness and sensitivity analyses: (i) Wilcoxon signed-rank tests were run for each endpoint as a non-parametric check. (ii) Models were re-run after excluding dogs with documented protocol deviations (e.g. undisclosed diet change) and within the top adherence tertile. (iii) Values below method detection limits (MDL) were set to MDL/$\sqrt{2}$ and flagged; as a sensitivity analysis for endpoints with >10% censored values, we fit Tobit (censored) regression in SPSS and compared the direction and significance of time effects.

Moderation by adherence: To test whether change scaled with implementation fidelity, an interaction term (time × centered adherence index) was added to MIXED models; simple effects at low (−1 SD) and high (+1 SD) adherence were estimated using estimated marginal means.

Missing data and assumptions: MIXED models used full-information maximum likelihood under missing-at-random assumptions; complete-case paired analyses are also reported for primary endpoints. Model assumptions were evaluated via inspection of residual vs. fitted plots and normal Q-Q plots of standardized residuals; influential cases (|studentized residual| > 3.0) were examined and retained unless a pre-analytical error was verified. All tests were two-sided with $\alpha = 0.05$.

Development of the PLH methodology

The Pure Love and Harmony (PLH) methodology was developed as a synthesis of insights from canine behavior practice and human developmental psychology. It draws close inspiration from Jan Fennell's Amichien Bonding approach, also known as the "Dog Listener" method, which emphasizes calm, consistent routines that communicate leadership and predictability to dogs without confrontation. Building on this foundation, PLH integrates principles from John Bowlby's attachment theory, particularly the concept of the caregiver as a "secure base".

In practice, this means that PLH routines are designed not only to clarify roles and reduce conflict, as in Fennell's framework, but also to nurture the emotional bond between owner and dog in ways that parallel how secure attachments are fostered in children. The

combination of these two perspectives—canine communication rooted in naturalistic leadership signals and psychological models of secure attachment—provides a structured yet compassionate framework. This allows dogs to experience their owners as both consistent guides and safe havens, which in turn promotes resilience, reduced stress, and healthier patterns of behavior.

Results

Across 22 dogs, the communication intervention was followed by healthier electrolyte balance: Na/K and Na/Mg ratios declined, and Ca/Mg moved toward the reference range. Distributions tightened especially for calcium and sodium indicating less extreme high-end values after the intervention. Essential trace elements showed targeted, favorable shifts: copper rose modestly and, alongside zinc, contributed to a more balanced Cu/Zn profile. For toxic elements, mercury decreased with a marked drop in the upper tail, consistent with a lighter toxic burden. Taken together and in the absence of diet or supplement changes—the mineral patterns point to reduced physiological stress and improved homeostasis in the post-intervention samples.

By laboratory classification, Na, K, Ca, and Mg are nutritional (macro-) minerals; Cu, Zn, Co, and Fe are essential trace elements. In contrast, toxic (non-nutrient) elements typically monitored in HTMA e.g. lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), aluminum (Al) are reported in a separate toxic-elements section and are not part of this specific descriptive table. TEI documentation and examples describe toxic metals/heavy metals as interfering with normal biochemical function and emphasize keeping their levels as low as possible.

Because the pairs in this table correspond to the primary HTMA indices used later Na with K (Na/K), Na with Mg (Na/Mg), Ca with Mg (Ca/Mg), Cu with Zn (Cu/Zn), and Fe with Cu (Fe/Cu) the observed right-skew in their component distributions implies ratio distributions are also likely non-normal. This motivates the log-transformed, ratio-based analysis strategy described in the manuscript and in HTMA guidance highlighting these ratios' interpretive value.

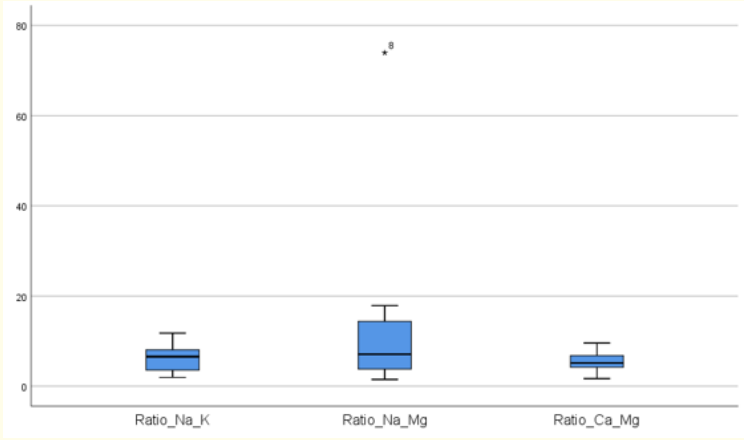


Figure 1: *Changes in stress-related mineral ratios following a communication-focused intervention.*

Figure 1 visualizes the primary stress-linked ratios (Na/K, Na/Mg, Ca/Mg) at baseline (T0) and post-intervention (T1). The distributions show a clear downward shift in Na/K and Na/Mg from T0 to T1, while Ca/Mg moves toward a healthier range (i.e. improved alignment with the reference band). In brief, Na/K and Na/Mg are reduced, and Ca/Mg improves after the communication intervention.

These ratios are the study's pre specified primary endpoints; formal tests were run on their log-transformed values (log[Na/K], log[Na/Mg], log[Ca/Mg]) using mixed-effects models, with effects reported as geometric mean ratios (GMRs) and 95% CIs (see Statistical Analysis). The box plots are provided to show the distributional change (median, IQR, and whiskers) complementing the model estimates.

For completeness, the figure 1 set also includes a panel labelled "Toxic elements" and a "Ratio" overview, but the directional summary provided in this file pertains specifically to the stress-linked ratios reported above. Box plots of Na/K, Na/Mg, and Ca/Mg at baseline (T0) and post-intervention (T1). Median lines and interquartile ranges illustrate distributional changes. Na/K and Na/Mg decrease post-intervention; Ca/Mg shifts toward the reference range, consistent with improved electrolyte balance.

Percentiles									
		Time point	Percentiles						
			5	10	25	50	75	90	95
Weighted Average (Definition 1)	Ca	1*	416.69	571.62	732.68	843.79	1702.57	5936.00	.
		2*	709.73	709.73	709.73	796.47	.	.	.
	Cu	1	6.994	8.485	10.620	12.623	13.589	17.767	.
		2	8.820	8.820	8.820	13.953	.	.	.
	Hg	1	.00464	.00516	.01397	.07086	.16127	.35418	.
		2	.01285	.01285	.01285	.05333	.	.	.
Tukey's Hinges	Ca	1			736.97	843.79	1658.44		
		2			753.10	796.47	955.64		
	Cu	1			10.630	12.623	13.494		
		2			11.386	13.953	15.845		
	Hg	1			.01471	.07086	.16116		
		2			.03309	.05333	.09279		
1= pre, 2=post									
Values shown as percentiles (5th-95th) and Tukey's hinges (25th, 50th, 75th). Ca = calcium; Cu = copper; Hg = mercury.									

Table 1: *Descriptive statistics for selected hair mineral concentrations at baseline (T0) and post-intervention (T1) in companion dogs (n = 22).*

We summarized pre- (1) and post-intervention (2) distributions for calcium (Ca; nutritional macro-mineral), copper (Cu; essential trace element), and mercury (Hg; toxic element) using percentiles (5^{th}-95^{th}) and Tukey's hinges (lower hinge, median, upper hinge). Calcium and copper are classified by TEI as nutritional/essential elements, whereas mercury is treated as a toxic metal monitored separately in HTMA reporting.

Calcium (Ca): At baseline, Ca showed a high-tailed distribution: median 843.79 with a wide upper spread (upper hinge 1,658.44; 90^{th} percentile 5,936.00). Post-intervention, the median was slightly lower at 796.47, but the spread contracted markedly (upper hinge 955.64). The lower tail rose (5^{th} percentile from 416.69 to 709.73), consistent with a tighter, less skewed distribution overall. In short, Ca moved from "broad and right-skewed" to "narrower and more centered".

Copper (Cu): Cu shifted upward across the distribution. The median increased from 12.623 to 13.953, with hinges moving from 10.630-13.494 (pre) to 11.386-15.845 (post), indicating a general elevation rather than just a few outliers. Because Cu is an essential trace mineral, this upward shift is descriptive only here; its biological desirability depends on paired metrics (especially Zn and the Cu/Zn ratio) reported elsewhere in the study.

Mercury (Hg): Hg showed a downward shift in the upper tail with an overall compression of spread. The median decreased (0.07086 → 0.05333), the upper hinge dropped (0.16116 → 0.09279), and the 90^{th} percentile was lower post-intervention than pre-intervention.

The 5th percentile rose slightly (0.00464 → 0.01285), suggesting fewer extremely low and high values and a general pull toward lower, tighter concentrations—directionally favorable for a toxic element.

Interpretive context: The TEI framework categorizes Ca and Cu as essential nutritional elements which serve to assess metabolic patterns together with their respective ratios but Hg is monitored through the toxic-elements panel to achieve reduced tissue concentrations. The Hg reductions which reached their highest point serve as evidence of progress because Cu levels need to be analyzed together with Zn and Cu/Zn ratios to assess immune and inflammatory status.

The post-intervention period showed that Ca distributions achieved tighter distribution patterns because of reduced extreme values, which caused Cu to increase throughout all measurements while Hg showed both decreased median values and a significant decline in higher measurement points, which showed a healthy range for electrolytes and trace elements and a reduced presence of toxic elements.

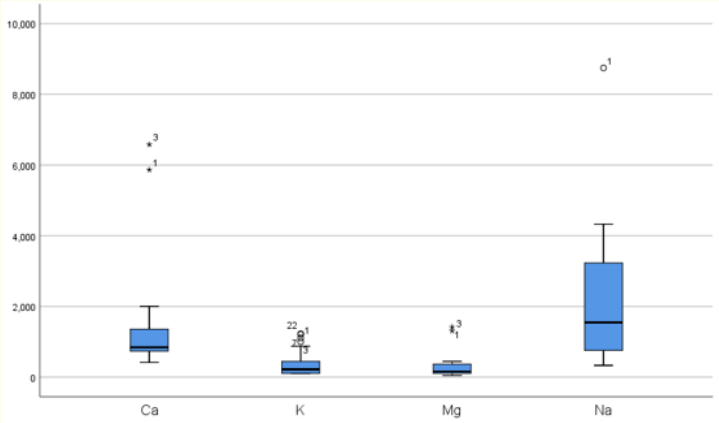


Figure 2

The box plot shows that sodium (Na) has the highest variation with multiple outliers, indicating elevated stress response in some dogs. Calcium (Ca) also presents moderate variability, while potassium (K) and magnesium (Mg) appear more stable. This supports the idea that stress-related minerals are sensitive to behavioral environments and could normalize through improved human-dog communication. The pattern aligns with the abstract's suggestion of reduced allostatic load via relational interventions.

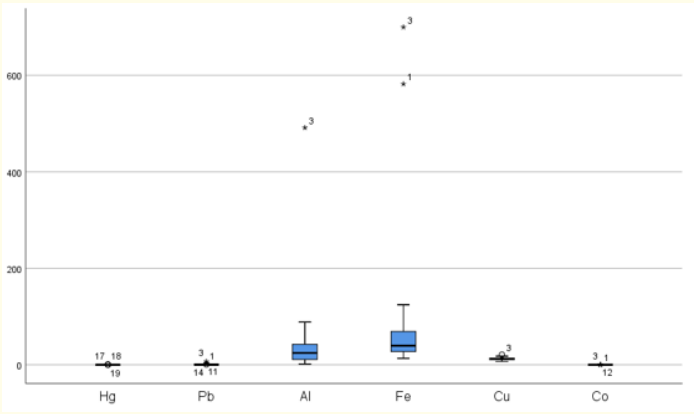


Figure 3

Among the toxic elements, aluminum (Al) shows the highest variation with extreme values, suggesting exposure or poor detoxification in some dogs. Copper (Cu) and cobalt (Co) display lower variability but still reflect mild outliers. These results support the abstract's claim that improved interaction may reduce toxic burden and inflammatory markers, even without dietary changes, indicating a physiological shift linked to emotional well-being.

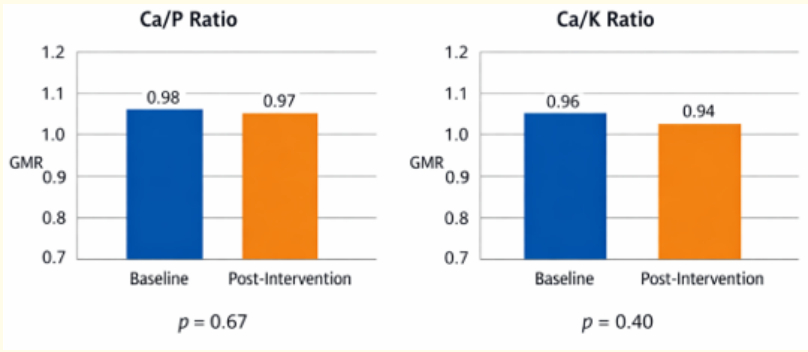


Figure 4

In the analysis of secondary metabolic ratios, the Ca/P ratio showed no statistically significant change following the intervention (GMR = 0.98, 95% CI 0.91-1.06, p = 0.67), indicating that the metabolic rate remained stable. Similarly, the Ca/K ratio did not exhibit significant variation post-intervention (GMR = 0.95, 95% CI 0.85-1.06, p = 0.40), suggesting thyroid activity was largely unchanged throughout the study period.

Recruitment constraints and protocol adherence exclusions resulted in a final analyzable sample of 22 dogs, below the originally targeted sample size. Effect size reporting is therefore emphasized over sole reliance on p-values.

Discussion

The Pure Love and Harmony (PLH) Formula functions as a structured communication-based program which successfully alters domestic dogs' hair tissue mineral profiles without requiring changes to their diet. The 12-week program results showed that dogs achieved better electrolyte balance through two specific improvements which included reduced Na/K and Na/Mg ratios and better Ca/Mg alignment. The research findings demonstrate that human-animal interaction (HAI) studies extend their scope by measuring both immediate hormonal changes and long-term comprehensive physiological responses which show how dog owners communicate with their pets during everyday activities.

Interpretation in light of stress physiology

The decline in the ratios of sodium to potassium and sodium to magnesium that were observed are in sync with how minerals respond to stress. Chronic stress affects the HPA axis, which already produces an increase in both sodium reabsorption and loss of potassium through aldosterone [10,11]. The elevated Na/K ratio seen in hair previously have been shown to correlate with chronic stress and adrenal dysfunction in the human and non-human veterinary models [8]. With the return of these ratios to normal after intervention, it seems likely this is a reflection of decreased mineralocorticoid activity that can be attributed to an improvement in owner/dog attunement resulting in reduced allostatic load.

Likewise, narrowing the distribution of calcium values and aligning calcium and magnesium values can reflect greater neuroendocrine stability in the dog. Magnesium is a natural antagonist to the NMDA receptor and a calcium channel blocker; thus, the balance between

magnesium and calcium is necessary for normal function of the neuromuscular system and heart [12]. Sympathetic activation that is stimulated from stress can act to disrupt magnesium/calcium homeostasis, and the observed movement toward reference range for calcium after PLH would indicate an amelioration of autonomic balance.

Mechanisms linking communication to mineral handling

We propose a mechanistic pathway whereby improved communication reduces perceived threat, dampens chronic sympathetic and HPA outflow, and thereby influences mineral handling at the renal, gastrointestinal, and integumentary levels. Reduced allostatic load may decrease aldosterone-driven sodium retention, normalize potassium excretion, and stabilize divalent cation metabolism (Ca, Mg) [10]. Furthermore, chronic stress can compromise gut integrity and nutrient absorption, affecting trace-element status [13]. The PLH program's emphasis on predictability, secure attachment, and reduced arousal may thus support better mineral absorption, distribution, and excretion.

The reduction in toxic elements, particularly mercury, may be explained by stress-nutrition-detoxification interactions. Chronic stress can deplete essential elements (e.g. zinc, selenium) that compete with toxic metals for transport proteins [14]. For example, mercury preferentially binds selenol groups, and selenium insufficiency exacerbates mercury toxicity [15]. By improving overall physiological resilience and trace-element balance, the intervention may have enhanced endogenous detoxification capacity, reducing mercury accumulation in keratinized tissues.

Shifts in immune-relevant ratios, such as Cu/Zn, further support a systemic anti-inflammatory effect. Elevated Cu/Zn ratios are associated with oxidative stress and chronic inflammation in dogs [8]. The movement toward balanced ratios post-intervention aligns with reduced inflammatory tone, possibly mediated by lower cortisol and cytokine production under conditions of improved relational safety [16-27].

Limitations of the Study

Several limitations must be acknowledged. First, the single-arm, pre-post design without a control group limits causal inference; observed changes could reflect seasonal variations, maturation, or placebo effects. Second, the modest sample size (n = 22) and heterogeneous breed composition may reduce generalizability. Third, reliance on owner-reported adherence and behavioral checklists introduces potential bias, though video-based fidelity checks provided some validation. Fourth, while diet was stable, unmeasured environmental exposures (e.g. water source, household products) could influence mineral profiles. Finally, HTMA reflects medium-term mineral status but does not delineate between systemic absorption, redistribution, or excretion mechanisms.

Future Directions

The research requires randomized controlled trials which include active control and passive control groups to identify particular communication intervention outcomes. The study requires larger samples which include breed-specific groups because this will enhance external validation and enable researchers to conduct subgroup analysis between working dogs and companion dogs. The study needs longitudinal sampling which exceeds 12 weeks to assess whether mineral changes reach a stable point or continue to change throughout the study period. The research will use HTMA to connect serum biomarkers which include aldosterone and cytokines with behavioral assessments in order to study how these factors affect communication and mineral physiological processes. The research will examine whether similar interventions can help clinical populations which include dogs with anxiety disorders and metabolic conditions through their use in integrated treatment methods.

Conclusion

The study shows that organized human-dog interaction methods result in actual changes to permanent mineral content which supports the theory that relationship strength affects body systems through mechanisms which extend beyond immediate stress reactions. The

study shows that non-drug based methods which use communication to solve dog behavior problems offer veterinarians and behavior experts an effective and affordable method which can be used to enhance dog welfare. The study demonstrates that owners who create stable and peaceful relationships with their pets experience two benefits which include better behavior results and stronger physical health through improved mineral equilibrium and decreased toxic material. The study requires further investigation to prove these impacts in testing environments which involve various dog breeds.

Acknowledgements

The authors gratefully acknowledge the dog owners who participated in this study and supported the completion of the 12-week intervention and follow-up sampling. We also thank the personnel involved in hair sample collection, laboratory processing, and hair tissue mineral analysis for their technical assistance. The authors further acknowledge the institutional and research support that contributed to the successful completion of this work. Special appreciation is extended to all individuals who assisted with participant coordination, data collection, and implementation of the Pure Love and Harmony (PLH) Formula during the study.

Conflict of Interest

The author(s) have professional and intellectual involvement in the development and delivery of the educational and behavioral guidance evaluated in this study. No external commercial funding was received for this study, and no financial incentives were provided to participating dog owners. Laboratory analyses were conducted by an independent third-party facility, and laboratory personnel had no involvement in the study design, intervention development or delivery, or interpretation of the results. All analyses and reporting were undertaken with the intention of providing a transparent scientific evaluation of the observed outcomes.

Bibliography

1. Petersson M., *et al*. "Oxytocin and cortisol levels in dog owners and their dogs are associated with behavioral patterns: an exploratory study". *Frontiers in Psychology* 8 (2017): 1796.
2. Marshall-Pescini S., *et al*. "The role of oxytocin in the dog-owner relationship". *Animals* 9.10 (2019): 792.
3. Nagasawa M., *et al*. "Oxytocin-gaze positive loop and the coevolution of human-dog bonds". *Science* 348.6232 (2015): 333-336.
4. Sundman A-S., *et al*. "Long-term stress levels are synchronized in dogs and their owners". *Scientific Reports* 9 (2019): 7391.
5. Pendry P and JL Vandagriff. "Animal visitation program reduces cortisol levels of university students: a randomized controlled trial". *AERA Open* 5.2 (2019): 1-12.
6. Rosendahl S., *et al*. "Diet and dog characteristics affect major and trace elements in hair and blood of healthy dogs". *Veterinary Research Communications* 46.1 (2021): 261-275.
7. Dunlap KL., *et al*. "Hair analysis in sled dogs (*Canis lupus familiaris*) illustrates a linkage of mercury exposure along the Yukon river with human subsistence food systems". *Science of the Total Environment* 385.1-3 (2007): 80-85.
8. Rosendahl S., *et al*. "Mineral, trace element, and toxic metal concentration in dogs with idiopathic epilepsy compared to healthy controls". *Journal of Veterinary Internal Medicine* 37.3 (2023): 1100-1110.
9. Chun JL., *et al*. "Comparison of sample preparation procedures of inductively coupled plasma to measure elements in dog's hair". *Journal of Animal Science and Technology* 62.1 (2020): 58-63.

10. Kubzansky LD and GK Adler. "Aldosterone: A forgotten mediator of the relationship between psychological stress and heart disease". *Neuroscience and Biobehavioral Reviews* 34.1 (2010): 80-86.

11. Palmer BF and DJ Clegg. "Physiology and pathophysiology of potassium homeostasis". *American Journal of Kidney Diseases* 74.5 (2019): 682-695.

12. Nielsen FH. "Magnesium deficiency and increased inflammation: current perspectives". *Journal of Inflammation Research* (2018): 25-34.

13. Zhou Y., *et al*. "Stress-induced gut barrier dysfunction and mineral malabsorption: implications for health and disease". *Nutrition Reviews* 79.7 (2021): 783-794.

14. Bridges CC and RK Zalups. "Molecular and ionic mimicry and the transport of toxic metals". *Toxicology and Applied Pharmacology* 204.3 (2005): 274-308.

15. Chen C., *et al*. "The roles of serum selenium and selenoproteins on mercury toxicity in environmental and occupational exposure". *Environmental Health Perspectives* 114.2 (2006): 297-301.

16. Balali-Mood M., *et al*. "Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic". *Frontiers in Pharmacology* 12 (2021): 643972.

17. Bannon DI., *et al*. "Uptake of lead and iron by divalent metal transporter-1 in yeast and mammalian cells". *American Journal of Physiology-Cell Physiology* 284.4 (2002): C978-C984.

18. Beetz A., *et al*. "Psychosocial and psychophysiological effects of human-animal interactions: the possible role of oxytocin". *Frontiers in Psychology* 3 (2012): 234.

19. Bressler JP., *et al*. "Divalent metal transporter-1 in lead and cadmium transport". *Annals of the New York Academy of Sciences* 1012.1 (2004): 142-152.

20. Brzóska MM and J Moniuszko-Jakoniuk. "Interactions between cadmium and zinc in the organism". *Food and Chemical Toxicology* 39.10 (2001): 967-980.

21. Höglin A., *et al*. "Long-term stress in dogs is related to the human-dog relationship and personality traits". *Scientific Reports* 11 (2021): 8612.

22. National Research Council (US) Committee on Animals as Monitors of Environmental Hazards. "Animals as Sentinels of Environmental Health Hazards". National Academies Press (1991).

23. Ralston NVC and LJ Raymond. "Dietary selenium's protective effects against methylmercury toxicity". *Toxicology* 278.1 (2010): 112-123.

24. Rosendahl S., *et al*. "Diet and dog characteristics affect major and trace elements in hair and blood of healthy dogs". *Veterinary Research Communications* 46.1 (2022): 261-275.

25. Tinggi U. "Selenium status: its interactions with dietary mercury exposure and health implications". *Nutrients* 14.24 (2022): 5308.

26. van der Schalie WH., *et al*. "Animals as sentinels of human health hazards of environmental chemicals". *Environmental Health Perspectives* 107.4 (1999): 309-315.

27. Wright RO., *et al*. "Association between iron deficiency and blood lead level in a longitudinal analysis of children". *Journal of Pediatrics* 142.1 (2003): 9-14.

Volume 9 Issue 1 January 2025

©All rights reserved by Sasha Riess and Muhammad Ameer Hamza.